

part of a defense reaction set up in adjacent normal bone against invasion by tumor tissue

METHODS

Serum phosphatase was determined by a modification of the Bodansky method which has been described in detail elsewhere (10). Estimation of the icteric index is a part of this procedure. Acid serum phosphatase was determined at pH 6.4 (11). The method was the same as for alkaline phosphatase except that the veronal buffer was omitted from the substrate. By the method used, normal values for alkaline serum phosphatase are 17 to 50 units, values above 45 units being rare and the average being 30 units. The acid serum phosphatase seldom exceeds 5 per cent of the alkaline phosphatase in normals or in patients with primary bone disease, but is markedly elevated in cases of carcinoma of the prostate metastatic to bone. Tissue phosphatase was determined by a modification (9) of the method of Franseen. Sodium beta glycerophosphate was used as substrate throughout. As most of the patients were seen in the out-patient department, the blood samples were usually obtained in the non-fasting condition.

Three types of cases were chosen for examination

A Patients without evidence of bone disease.

B Patients with symptoms suggestive of bone involvement but with negative films. In some of these patients the symptoms were probably, but not certainly, due to coexistent arthritis.

C Patients with bone lesions demonstrable roentgenographically.

It should be emphasized that these patients were selected with a view to possible bone disease. Only enough cases without bone symptoms were examined to give an adequate control group. An unselected series of patients from the same clinic would include a much larger percentage without symptoms referable to the bones.

All the patients reported were adults. A few children were also examined but are not included here, since it is very difficult to evaluate serum phosphatase findings in growing individuals, owing to the wide range of normal variation.

RESULTS

Results for serum phosphatase are summarized in Table I. Values above 50 units per 100 cc are considered abnormal. Serial observations were made on many of the patients over periods ranging from a few months to nearly two years. During this time some patients who had at first no evidence of bone disease developed pain in the bones. Others who had symptoms suggestive of bone involvement without roentgenographic changes later showed frank lesions. Whenever such a change in the status of the patient with

TABLE I
Summary of the serum phosphatase readings of 115 patients with lymphomatoid disease

Diagnosis	Cases without bone symptoms		Cases with bone symptoms but with normal x rays		Cases with bone lesions shown roentgenographically	
	Total number	Number with elevated P-ase	Total number	Number with elevated P-ase	Total number	Number with elevated P-ase
Hodgkin's disease	11	3	36	21	19	14
Lymphosarcoma	13	4	8	5	9	3
The leukemias	15	4	11	1	3	1
Miscellaneous	1	1	4	3	3	2
Total	40	12 or 30 per cent	59	30 or 51 per cent	34	20 or 59 per cent

respect to evidence of bone disease occurred, another record was made in the appropriate column. The table therefore contains 134 entries relating to 115 patients.

Determinations of total serum calcium and of serum inorganic phosphorus were made on all cases but are not recorded in the table as no striking deviations from normal were observed. A slight elevation of serum inorganic phosphorus was found in about a third of the Hodgkin's cases and may possibly be of significance. In the leukemias the total serum calcium averaged a little low, but this was not related to the presence of a hemorrhagic tendency and was probably due to the poor nutrition of many of these patients.

The section of the table entitled "Miscellaneous" includes 3 cases with a tendency both to polycythemia and leukemia, and 1 each of giant follicular lymphoma, non-specific lymphadenitis, mycosis fungoides, probably aleukemic lymphatic leukemia, and aleukemic myeloid leukosarcoma. In most of the patients with leukemia and polycythemia, the diagnosis was established by blood examination alone. In all the others except 3 the diagnosis was verified by biopsy. In the group of 26 cases of leukemia, 19 were of the lymphatic type, 4 of the myelogenous, and 3 of the pseudo-leukemic.

It is evident at once from inspection of the table that an elevation of serum phosphatase is very common in the lymphomatoid diseases. It

occurred in over half the cases with known bone involvement or with symptoms suggestive of bone involvement, and also in more than a quarter of the cases in which there was no reason to suspect that the bones were affected. When the different diagnostic groups are examined separately, it is seen that for the 66 determinations on 53 cases of Hodgkin's disease the relation between elevation in serum phosphatase and evidence of bone disease is very close. The actual values could not be presented in the table, but it may be stated here that in Hodgkin's cases with severe bone symptoms the elevation of serum phosphatase was often marked, the readings being above 15 units in 9 patients and above 35 units in 2. The histories of some of these patients will be examined in detail later.

The miscellaneous group is too small to be of much significance, but the relation of changes in serum phosphatase to bone symptoms is fairly close.

The patients with lymphosarcoma present many puzzling features, as an elevation of serum phosphatase is encountered as often in the patients without evidence of bone involvement as in those with demonstrable lesions. In the group of patients with leukemia well-defined bone lesions were rare. Elevations of serum phosphatase were small, only one value exceeding 6.5 units being found. They occurred infrequently and bore little relation to the presence of symptoms referable to the bones.

Because of the lack of correlation between the presence of elevated serum phosphatase values and evidence of bone disease in lymphosarcoma and the leukemias, the question presents itself whether the excess phosphatase in the serum of many of these patients comes from the bones or from some other tissue. The latter is an important possibility, as many patients with lymphomatoid disease have large masses of abnormal tissue and radical disturbances of the vital metabolic processes.

An increase in metabolic rate is common in these patients. It is not associated with symptoms of hyperthyroidism and its exact cause is obscure. Little evidence is available at present regarding the effect of metabolic rate on serum phosphatase, although the phosphatase appears to

be somewhat elevated in hyperthyroidism (12). We have not determined the basal metabolic rate on the cases presented here, but we observe that gross abnormalities in serum phosphatase are encountered least often in the leukemias where an accelerated metabolism is most common. In several patients with Hodgkin's disease there was no relation between the serum phosphatase readings and the presence of fever. We feel, therefore, that while an increase in the metabolic rate may explain why many patients with leukemia have serum phosphatase values in the upper normal range, it does not account for the markedly elevated phosphatase readings so often found in Hodgkin's disease.

The two most probable non-osseous sources of the excess phosphatase in the serum are the bile and the lymphomatoid tissue. None of the patients reported here had an elevated icteric index, so it is unlikely that there were significant amounts of bile phosphatase in the serum. While recent work (13) has shown that the serum phosphatase may remain elevated for a time after the icteric index has returned to normal following an attack of jaundice, further evidence has just been obtained in the course of an uncompleted investigation by one of us (H. Q. W.) which indicates that moderate liver dysfunction without jaundice does not produce significant changes in the serum phosphatase. We feel, therefore, that liver disease is not an important factor in our phosphatase findings, but cannot be definitely excluded in all cases.

Since it was found that the amounts of both acid and alkaline phosphatase in the lymphomatoid tissue were small, it does not seem likely that enzyme from this tissue was responsible for the high values found for alkaline serum phosphatase. Additional evidence on this point was obtained from determination of the acid phosphatase of the serum. This was done on 29 cases with an elevated alkaline phosphatase and 25 cases with a normal alkaline phosphatase. No abnormalities in the acid phosphatase were found. It is improbable that leakage of alkaline phosphatase to the serum would take place without corresponding leakage of acid phosphatase.

Although the above evidence does not favor a non-osseous origin for elevations of the serum

phosphatase, an attempt was also made to use statistical methods to discover whether there was a correlation between changes in phosphatase and the presence of extensive adenopathy or of enlargement of the liver or spleen. This method is not entirely satisfactory because lesions of liver, spleen, bones, and glandular systems cannot be considered as independent variables, and are likely to occur simultaneously late in the disease. Nevertheless, significant exceptions to this are often found, and the statistical study was therefore made. It showed that, for Hodgkin's disease, the only positive correlation was that between serum phosphatase and evidence of bone disease. The series of cases of lymphosarcomas and the leukemias were rather small to be significant but suggested that disease of the liver or spleen might be a factor in raising the serum phosphatase.

Since the white blood cells contain alkaline phosphatase, it is theoretically possible for enzyme from this source to raise the serum level. However, it was apparent at once that this was not an important practical factor. An elevated serum phosphatase was infrequent in the leukemias, and bore no relation to the white count when it did occur.

Although previous work has failed to show any evidence that radiation therapy has a direct effect on the serum phosphatase (14), the possibility of such an effect must be considered here. In the leukemias, particularly, the destruction of the white blood cells might result in the liberation of significant amounts of alkaline phosphatase into the serum, even though the intact cells do not affect the serum level. Many of our patients were under almost continuous treatment, so that it was not possible to obtain blood samples more than a few weeks after the last irradiation. Adequate observations on the effect of a single course of treatment were obtained on 4 cases, however, and are summarized below.

In the last case, irradiation resulted in a drop in the total white count from 92,400 to 17,200. The variations in phosphatase observed are within normal limits. This confirms our previous observations.

Irradiation of tissues other than the bones does not appear, therefore, to have a significant effect on the serum phosphatase in these patients.

Diagnosis	Phos phatase before treat- ment	Phos phatase after treat- ment	Area treated
	<i>Bodansky units</i>	<i>Bodansky units</i>	
Hodgkin's dis- ease	3 3	2 2	Supraclavicular and mediastinum
Hodgkin's dis- ease	4 3	4 7	Chest
Lymphosarcoma	4 8	3 6	Abdomen
Lymphatic leukemia	4 8	4 6	Neck and groins

From this discussion of the possible origins of serum phosphatase, it appears that in Hodgkin's disease all the evidence is in favor of an osseous origin for the excess phosphatase in the serum. This may also be true in lymphosarcoma and the leukemias, but in these diseases other possible sources of serum phosphatase have not been excluded.

PROTOCOLS

Some of the material summarized in the table will now be examined in more detail. In the group of 11 Hodgkin's patients who had no symptoms referable to the bones at the time of the first serum phosphatase determination, the majority had normal or high normal phosphatase readings.

The subsequent course of *Case 12*, who had an elevation of serum phosphatase which was unexplained at first, is of especial interest. This patient, a female aged 42, had been under treatment for proved Hodgkin's disease for two years. Her first phosphatase reading was slightly elevated at 5.9 units. At this time she was in good health but had a large supraclavicular mass. During the next three months she received two cycles of roentgen irradiation to the supraclavicular regions and mediastinum. At the close of treatment the adenopathy had regressed somewhat and the serum phosphatase was 57 units. Four months later she developed marked involvement of the head of the femur. She stated at this time that she had noted minor discomfort in this region for a period of one year, but she considered it too trivial to mention. The area was irradiated, but after completion of treatment the serum phosphatase was 91 units, and rose to 117 units during the next four months. At this time her general condition had deteriorated, there was massive adenopathy adherent to, and probably involving, the sternum, and a film showed that the femoral lesion was not healing. It is likely that in this case the bones were invaded early, but that it was not until the disease became very active that the bone disturbance was sufficient to cause serious symptoms.

In the group of cases of Hodgkin's disease with symptoms referable to bones, an elevation of the serum phosphatase was frequently encountered. In 3 of the patients who had serum phosphatase values within normal limits the symptoms were probably due to co-existent arthritis

In Case 22 (M. R.), a male aged 39 with a seven year history of Hodgkin's disease had serum phosphatase values which remained approximately constant at the somewhat elevated level between 6.6 and 8.2 units for a period of one year. Eighteen months after the first determination an exacerbation of bone symptoms was associated with a rise of phosphatase to 12.5 units. During this time the general condition was poor and the spleen was somewhat enlarged but there was little or no palpable adenopathy. The patient complained constantly of pain in the shoulder, lumbar spine, and pelvis, but repeated roentgenograms of these areas failed to show frank lesions although on several occasions a questionable alteration of density was noted. During the period of observation the patient received five cycles of roentgen ray treatment to the spleen and the suspected bones. It is difficult to escape the conclusion that in this patient Hodgkin's disease was present extensively in and about the bones and contributed to the marked impairment of the general health in the absence of external adenopathy. It was however controlled sufficiently by irradiation therapy to prevent the development of demonstrable lesions.

In Case 28 (J. H.) a male aged 31 with a four year history of Hodgkin's disease had some pain and tenderness in the spine but films were negative except for questionable wedging of the fifth lumbar vertebra. He was observed for nine months during which period his serum phosphatase rose irregularly from 8.4 units to the high value of 30.5 units. At different times he showed rather extensive axillary mediastinal and epigastric masses which were treated by three roentgen ray cycles. His general condition deteriorated and he died one month after the last observation. It is noteworthy that, except for the irradiation of the sternum incidental to the mediastinal therapy this patient received little roentgen ray treatment to the bones which are commonly involved in Hodgkin's disease. It is probable that he had extensive but nearly symptomless bone disease which progressed in parallel with disease elsewhere in the body.

In Case 31 (F. D.) a male aged 32 with a two-year history of Hodgkin's disease complained of bone pain but films of the lumbar spine, pelvis, and knees were essentially negative. His first serum phosphatase determination showed a moderately elevated value of 7.6 units. At this time he was in fair condition with no palpable adenopathy but he soon developed evidence of disease requiring a cycle of roentgen irradiation to the abdomen and treatment in the Heublein total irradiation unit. This failed to control the process so that three months after the first phosphatase determination he developed marked adenopathy and a temperature of 101. His serum phosphatase at this time was approximately

unchanged at 6.9 units. He received another course of Heublein therapy with little improvement, and in three months his serum phosphatase had risen to 12.0 units. Six weeks later a film of the dorsal spine showed definite sclerotic changes. After another treatment in the Heublein unit there was marked improvement in the adenopathy and general health, but the serum phosphatase rose to 17.4 units. There was coincident increase in back pain and films showed sclerosis of the dorsal vertebrae and decalcification of the pelvis. Roentgen irradiation of the dorsal spine resulted in relief of pain, but the serum phosphatase remained unchanged at 17.7 units, probably owing to the pelvic lesions. Seven months later the general condition was poor and the bone symptoms had become severe, with a corresponding rise of the serum phosphatase to 36.4 units. In this case, the serum phosphatase paralleled the evidence of bone involvement closely over a period of twenty-one months and showed no relation to the wide fluctuations in adenopathy.

In the group of Hodgkin's cases with bone lesions demonstrable roentgenographically, there is no close relation between the degree of elevation of serum phosphatase and the amount of new bone formed. Thus 2 patients with long standing and fairly extensive osteoplastic lesions had serum phosphatase values which ranged between 2.6 and 4.8 units, while in 3 other patients who showed osteolysis, the phosphatase was from 7.5 to 15.7 units. It may be significant that 1 of these eventually developed some osteoplasia. This is in contrast to the usual findings in carcinoma metastatic to bone where the serum phosphatase usually corresponds closely to the degree of osteoplasia.

In Case 38 (A. M.) a female aged 46 with a three-year history of Hodgkin's disease was observed for eighteen months. During this time she had little adenopathy but her general condition was only fair and disease activity showed itself at intervals in the form of herpes dermatitis and recurrent attacks of gastro-intestinal disturbance and fever. These were treated by cautious roentgen therapy and supportive measures. Nine months prior to the first phosphatase determination there had been a small osteolytic lesion of the third lumbar vertebra and symptoms suggestive of pelvic disease without roentgenographic changes. These became symptomless but the serum phosphatase at first examination was found to be definitely elevated at 7.0 units, and rose steadily to 12.6 units over a period of eleven months. This prompted a re-examination of the pelvis, and a large lesion of the left ilium was discovered. The region soon began to be painful and was irradiated with relief of symptoms. The serum phosphatase continued to rise to 22.0 units however and we anticipate that there will soon be evidence of disease in other bones.

In view of the uncertainty existing as to the significance of serum phosphatase changes in lymphosarcoma and the leukemias, the histories of patients with these diseases will not be considered in detail. It is of interest to note that in 3 cases of lymphosarcoma the disease was primary in bone and showed little or no extension to other organs. These may be examples of the primary reticulum cell sarcoma of bone recently described by Parker and Jackson (15) which these authors believe to be a separate entity. All of these patients had serum phosphatase values well within normal limits, resembling in this respect cases of plasma cell myeloma among the primary bone tumors. The 3 cases are from the clinic of Dr B. L. Coley of the Bone Service of this hospital.

In 3 cases in the miscellaneous group the presenting symptom was polycythemia. This was fairly well controlled at the time of first phosphatase determinations, but a leukemic tendency had also appeared. Adenopathy was slight or absent but splenomegaly was present. All these patients complained of pain and tenderness in the bones but roentgenograms failed to show any definite abnormalities except one small osteolytic lesion of the tibia in 1 case. In all cases the serum phosphatase was moderately elevated to from 62 to 90 units. This is interesting in view of the recent work of Sabin and coworkers (16) who have shown that, in rabbits which are anemic and leukopenic at birth, the demands on the bone marrow in early extrauterine life cause a proliferation of osteoclasts and enlargement of the marrow cavity. The similar proliferation of osteoclasts which occurs in hyperparathyroidism is known to be associated with an elevation of serum phosphatase. If the hyperplastic marrow of polycythemia also tends to stimulate the mechanism for enlargement of the marrow cavity at the expense of the adjacent bone, then an increase in serum phosphatase is to be expected.

COMMENT

The evidence obtained in this investigation lends support to the belief previously expressed by one of us (17) that bone involvement is much more common in the lymphomatoid diseases than the incidence of overt lesions would indicate. However, the process appears to differ radically

from that which takes place when the bones are the site of carcinoma metastases. Here the metastases, if untreated, progress regularly to a fatal termination, and the serum phosphatase, though higher in osteoplastic than in osteolytic disease, rises steadily with the spread of the metastases. In Hodgkin's disease, on the other hand, the progress of the bone lesions appears to be subject to relapses and remissions similar to those observed in other affected organs. As we have seen, the serum phosphatase of several of our cases showed over periods of many months somewhat elevated and fluctuating values associated with symptoms referable to the bones but unrelated to changes in adenopathy. Some of these patients eventually developed demonstrable bone changes, while others have not done so to date. If elevations of serum phosphatase indicate the presence of bone disease, as we believe they do, then these patients had bone pathology which made little progress over long periods. An important type of case is one in which the patient is obviously doing badly, yet has little adenopathy accessible to examination. When serum phosphatase readings remain persistently elevated in such patients, it seems likely that the disease is present principally in or about the bones. Finally, we have on several occasions observed a terminal rise of serum phosphatase to very high values. In these cases the disease had affected nearly every organ of the body, and had undoubtedly included the bones also.

As mentioned previously, the lymphosarcoma and leukemias present many unexplained anomalies, and further work will be necessary before the significance of serum phosphatase in these conditions can be made clear.

We may suggest a tentative explanation for the wide variations observed in the extent of serum phosphatase elevation between different cases of the same type. We believe that the elevation in phosphatase reflects a defense reaction on the part of the bones against invasion. If the defense is less than the invasive process, an osteolytic lesion will result. If the defense is greater than the invasive process, an osteoplastic lesion will result. If the two are equal, no demonstrable bone defect may develop, although symptoms may be present. Either or both may be slight or intense, but the degree of the serum

phosphatase elevation will be a measure of the defense mechanism only

SUMMARY

Serum phosphatase studies have been made on 115 patients with lymphomatoid disease, including 53 cases of Hodgkin's disease 28 of lymphosarcoma 26 of leukemia, and 8 of miscellaneous lymphomatoid disorders

The alkaline serum phosphatase was frequently elevated in Hodgkin's disease, but less often in lymphosarcoma and the leukemias Abnormalities in the acid serum phosphatase were not found.

Elevated serum phosphatase values occurred in many patients having symptoms referable to the bones, although no bone changes were demonstrable roentgenographically Some of these patients later developed demonstrable lesions

Bone changes probably occur much more frequently than the incidence of overt lesions indicates

A persistently elevated serum phosphatase in a case of Hodgkin's disease probably indicates invasion of bone

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PRECIPITIN STUDIES IN NEPHROSIS AND NEPHRITIS

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In a previous paper (1) measurements by the quantitative precipitin method were reported which demonstrated an immunologic difference between normal serum proteins and the serum proteins from a few patients during the edematous stage of nephrosis, the sera from the nephrotic patients failed to precipitate completely with specific antisera. Coincident with clinical improvement following diuresis both albumin and globulin regained their normal behavior. The serum proteins of a few nephritic patients did not show such marked failure to react.

Since the sera from nephrotic patients reacted incompletely with anti normal albumin and anti-normal globulin sera, it was suspected that the part of the protein which reacted had retained the serological characteristics of normal protein and that the remainder of the protein was changed in some peculiar fashion. The altered protein disappeared gradually during convalescence following diuresis. Hence it was assumed that during the edematous stage of nephrosis the serum proteins were mixtures of normal and altered protein with the altered protein usually predominating, after diuresis with clinical improvement, the altered protein decreased in favor of an increase of the normal protein.

This peculiar property of the serum proteins of nephrotic patients aroused curiosity on several points. Is the change in the serum protein present early in the course of the nephrosis? Can the amount of altered serum protein be correlated with any of the important clinical features of nephrosis such as superimposed infection, albuminuria, cholesteremia or edema? Can an arbitrary level of the altered protein be selected which will differentiate nephrotic from nephritic sera in order to secure more accurate diagnosis and prognosis in kidney disease in children? What is the nature of the altered protein? Is altered protein present in the sera of patients with diseases other than nephrosis and nephritis?

The purpose of this report is to present observations on a larger series of patients over a period of 3 years in an attempt to answer the above questions. Twenty three patients with nephrosis and 17 with nephritis have been studied. Frequent observations were made on 19 of the nephrotic patients until death occurred or recovery ensued, in the remaining 4 cases one observation was made for diagnostic purposes only, in 11 of the 17 nephritic patients prolonged studies were possible.

In Table I the analyses on the sera of 6 of the 23 patients with nephrosis are assembled, together with some of the pertinent clinical findings. The values obtained with the quantitative precipitin method are compared with the results by Howe's method, the difference between the two values was considered to represent the altered protein, that is, the protein which showed abnormal serological reaction. The technique used have been described previously (2). An examination of the data reveals that in each patient at some time during the course of the disease the amount of altered albumin was 40 per cent or more of the total albumin as determined by Howe's method, the globulin, on the other hand was unaffected in some patients and showed varying amounts of altered globulin in others. In 17 cases both altered albumin and altered globulin were found while in 5 cases the albumin only was affected.

1. Is the change in the serum proteins present early in the course of nephrosis? It is important to determine whether the change in the serum protein appears early enough in the course of nephrosis to be of primary etiologic significance, or whether it appears after prolonged albuminuria or persistent malnutrition has presumably strained the mechanism for the synthesis of serum protein. If it could be demonstrated that the altered protein were present before albuminuria had occurred or before severe malnutrition were a complicating factor, it would be justifiable to accord

TABLE I

Analyses of sera from cases of nephrosis Comparison of Howe's method and the precipitin method

Patient age date	Albumin			Globulin			Albumin- uria	Edema	Chole- sterol	Comment
	Howe	Precip- itin normal	Al- tered	Howe	Precip- itin normal	Al- tered				
	grams per 100 cc			grams per 100 cc					mgm per 100 cc	
Anna F 8 years										First attack of edema Edema present 3 months Upper respiratory infection
October 22, 1935	1 35	0 81	0 54	2 12	0 67	1 45	++++	++++	842	
February 10, 1936	0 85	0 09	0 76	2 14	0 80	1 34	++++	++++	641	
February 19, 1936	1 72	1 09	0 63	2 60	4 30	0	+	±	502	Diuresis in progress
March 5, 1936	2 55	2 47	0 08	2 72	4 67	0	+	0		
March 11, 1936	3 08	3 24	0	2 84	3 59	0	+	0	363	
April 8, 1936	3 41	3 28	0 13	2 52	2 30	0 22	+	0	211	
May 6, 1936	3 76	3 81	0	2 42	2 31	0 11	++	0	227	
July 14, 1936	4 26	4 50	0	2 50	2 35	0 15	+	0	203	
September 22, 1937	5 23	4 94	0 29	2 13	2 70	0	0	0	195	Recovered
C C 3 years										First attack of edema Edema present 4 days Upper respiratory infection Diuresis in progress
April 24, 1936	1 68	1 30	0 38	2 48	3 02	0	++++	++++	421	
April 30, 1936	2 57	2 22	0 35	2 70	1 55	1 25	+	±		
May 13, 1936	3 51	3 70	0	2 11	1 94	0 17	±	0	332	
June 3, 1936	3 44	3 29	0 15	2 21	2 05	0 16	+	0	246	
June 17, 1936	3 11	3 41	0	2 39	1 55	0 84	++	0	237	
October 3, 1936	1 95	1 27	0 68	2 47	3 75	0	++++	++++	490	2d attack edema Upper respira- tory infection
October 13, 1936	2 47	1 81	0 66	2 43	3 32	0	++++	+	543	Diuresis in progress
January 8, 1937	1 06	0 63	0 43	2 55	2 43	0 12	++++	++++	678	3d attack edema Septicemia. Pneumococcus 6
January 15, 1937	1 70	0 42	1 28	2 14	2 40	0	++++	++++	711	Metastatic abscesses Pneumo- coccus 6
February 20, 1937	4 42	4 58	0	2 54	2 76	0	±	0	273	
November 3, 1937	4 06	3 22	0 84	1 69	1 62	0 07	++	0	292	
January 12, 1938	4 36	4 64	0	1 81	2 60	0	+	0	227	
August 9, 1938	2 30	1 73	0 57	1 74	2 24	0	++++	±	534	Upper respiratory infection
August 31, 1938	2 92	2 68	0 24	2 09	3 13	0	++++	±		
October 12, 1938	1 62	0 86	0 76	2 44	2 47	0	++++	++++	912	4th attack edema Upper re- spiratory infection
October 19, 1938	1 01	0 83	0 18	2 85	3 47	0	++++	+++	1066	
October 28, 1938	1 11	0 56	0 55	2 45	1 73	0 72	+++	++++	827	
H S 3 years										2d attack of edema Edema present 2 months Upper respiratory infection Otitis media
May 28, 1935	1 77	0 84	0 93	2 02	0 27	1 75	++++	++++	411	
June 5, 1935	1 51	0 27	1 24	2 26	1 05	1 21	++++	++++	378	
June 12, 1935	1 63	0 68	0 95	2 32	1 03	1 29	++	++++	553	
July 29, 1935	1 97	1 26	0 71	3 68	4 01	0	++	±	178	Bilateral otitis media Multiple abscesses. Pneumococcus 6
September 25, 1935	4 60	3 90	0 70	2 38	1 75	0 63	+	0		Diuresis in progress
January 29, 1936	0 83	0 59	0 24	2 36	0 35	2 01	++++	++	379	Readmission Upper respiratory infection
February 7, 1936	1 31	0 60	0 71	2 35	2 60	0	++++	++++	665	
February 18, 1936	1 09	0 17	0 92	1 96	1 76	0 20	++++	++++	693	Septicemia and peritonitis Pneu- mococcus 23 Died February 23, 1936

TABLE I—Continued

Patient, age, date	Albumin			Globulin			Albuminuria	Edema	Cholesterol	Comment
	Howe	Precipitin normal	Altered	Howe	Precipitin normal	Altered				
	grams per 100 cc.			grams per 100 cc.					mgm per 100 cc.	
J J 2½ years										
October 27 1937	1.35	0.58	0.77	2.31	2.02	0.29	++++	++++	892	First attack of edema Edema present 8 days.
November 15 1937	1.67	0.50	1.17	2.17	2.84	0	+	++++	635	Upper respiratory infection Bronchopneumonia Pneumococcus 10
November 26 1937	2.56	1.00	1.56	2.74	5.37	0	+	±	410	Diuresis in progress
December 1 1937	3.12	2.08	1.04	3.11	4.96	0	+	0	350	
December 13 1937	4.14	4.06	0.08	2.51	5.00	0	0	0	346	
September 9 1938	5.00	5.12	0	1.86	1.97	0	0	0	248	Readmission Tonsillectomy
October 10 1938	4.11	3.68	0.43	2.12	2.81	0	++++	0	333	2d attack edema Upper respiratory infection.
October 14 1938	3.35	2.86	0.49	2.35	2.48	0	++++	0	357	Mild upper respiratory infection
October 19 1938	2.72	2.25	0.47	1.92	1.93	0	++++	0	414	
November 1 1938	1.99	1.23	0.76	2.55	3.72	0	++++	0	525	
November 8 1938	2.28	1.45	0.83	2.23	2.73	0	++	±	590	
November 21 1938	2.70	2.01	0.69	2.41	2.99	0	+	±	590	
December 2 1938	3.32	2.82	0.50	2.13	1.94	0.19	±	0	552	
December 17 1938	4.16	3.87	0.29	2.03	2.50	0	±	0	333	
L G 2 years										
October 28 1935	1.98	0.87	1.11	2.73	0.60	2.13	+	+++	611	First attack of edema. Edema present 6 days.
November 11 1935	1.83	0.89	0.94	2.61	0.47	2.14	++++	+++	758	Mild upper respiratory infection Died 6 months later
J F 5 years										
June 22 1934	1.17	0.20	0.97	2.60	0.88	1.72	++++	++++	689	3d attack of edema.
June 27 1934	1.01	0.26	0.75	2.71	1.03	1.68	++++	++++	718	Edema present 8 months
July 9 1934	1.27	0.31	0.96	2.81	1.02	1.79	++++	++++	668	Upper respiratory infection
July 14 1934	1.30	0.27	1.03	2.30	0.84	1.46	++++	+++	599	
October 8 1934	0.91	0.22	0.69	2.31	1.17	1.14	++++	++++	475	
December 13 1934	2.75	2.33	0.42	2.57	1.76	0.81	+	0	378	Hemolytic streptococcus infection
January 28 1935	3.10	2.63	0.47	2.09	1.48	0.61	+	0	496	
February 25 1935	2.86	2.50	0.36	2.20	1.10	1.10	+	0		
May 13 1935	3.87	3.56	0.31	2.24	1.07	1.17	+	0		Died June 11 1936 Peritonitis and septicemia. Pneumococcus 20

the alteration in the protein a significant etiologic role.

Of the 23 nephrotic patients only 5 were admitted early enough to provide information on this point. In the 5 patients edema had first been noted from 4 to 21 days preceding admission to the hospital for an initial attack of nephrosis. In no patient was severe infection present either at the onset of symptoms or on admission. In the remaining 18 children edema had been present at least 1 month before admission. In 3 patients studies were made 6, 8 and 21 days after the onset of edema and in all 3 the derangement of the serum proteins had already developed to the maximum extent. The data on 2 of these pa-

tients L. G. and J. J. are included in Table I. It is significant that in 2 of these patients edema and albuminuria were likewise maximal on admission and persisted for some time. In the third patient (L. G.) however the albuminuria was slight on entry 6 days after edema had appeared, yet the serum protein derangement was maximal. In contrast in 2 patients in whom studies were made 4 and 14 days after the onset of edema the serum proteins showed changes of lesser degree on admission. Patient C. C. reported in Table I, observed 4 days after the onset of edema presented milder changes in the serum proteins, yet the clinical findings and were similar to those found in patient

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cluded in Figure 3 is a diagram of the relation of normal serum albumin to total serum cholesterol. It is clear that normal values for total cholesterol are always associated with normal levels of normal serum albumin. The cholesterol analyses included estimations of the free and esterified fractions after the method of Schoenheimer and Sperry (3). Frequently in nephrosis

rapidly while the regeneration of the serum protein requires considerable time. The altered protein, cholesteraemia, and albuminuria likewise decrease gradually. Hence, high correlations between these manifestations cannot be expected. It was futile to speculate on the possibility of a correlation between the altered globulin and edema and the other pertinent clinical findings of

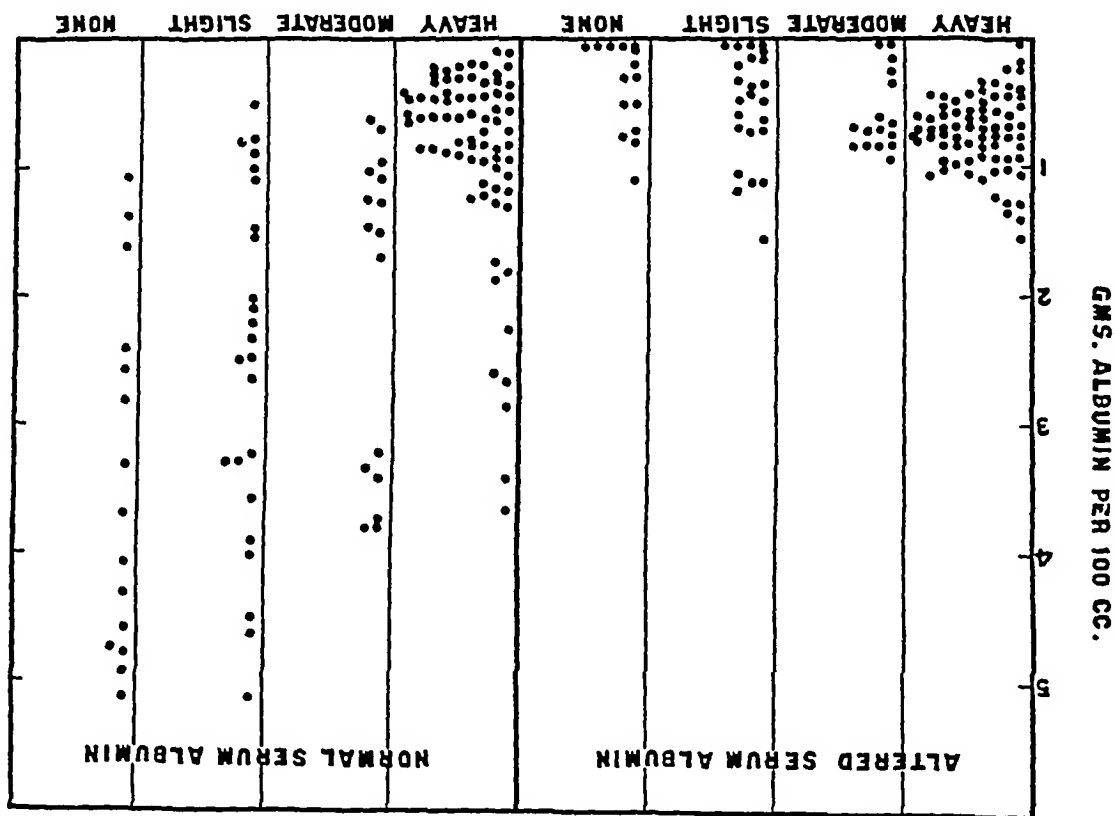


Fig 2 RELATION OF ALTERED AND NORMAL SERUM ALBUMIN TO ALBUMINURIA

the ratio of cholesterol ester to cholesterol is moderately low. Previously (4) it was found that a low ratio indicates either hepatic disease or infection and is independent of the amount of total cholesterol. When the ratios are plotted against the levels of total cholesterol (Figure 4), it is clear that the lowered ratios found in nephrotic sera are likewise not associated with the high values of total cholesterol. Studies of the natural history of nephrosis have shown that following diuresis clinical and laboratory findings return slowly but not simultaneously to the normal level. The edema disappears quite

nephrosis, because in some patients no altered globulin could be demonstrated during the entire illness. There was no difference clinically between the cases which showed altered globulin and those in which it was never demonstrated. It is possible that the failure to demonstrate altered globulin may be explained by a curious reaction of regenerating globulin to the specific anti-serum. Under circumstances in which there is reason to believe that new globulin is being formed in response to infection with the production of antibodies, or when new serum protein is being rapidly regenerated following a depletion,

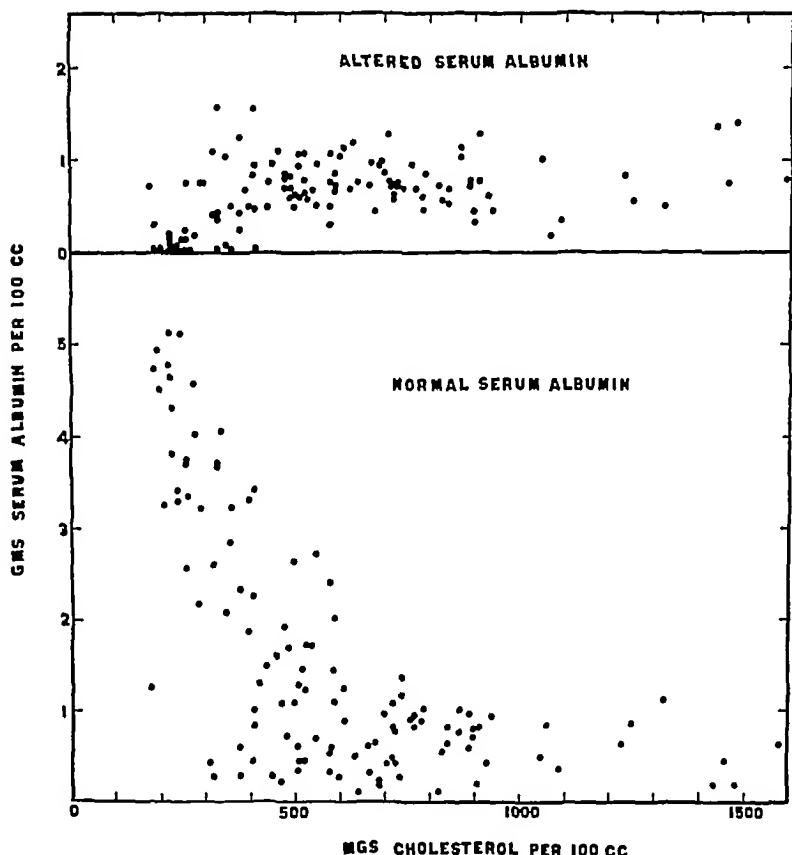


FIG. 3 RELATION OF ALTERED AND NORMAL SERUM ALBUMIN TO SERUM CHOLESTEROL

a disturbance in the distribution of globulin sub-fractions, as determined by Howe's method will be reflected in a high precipitin value. As the stimulus to infection or regeneration recedes the globulin sub-fractions gradually revert to normal and then the precipitin values are also normal. Such a series of changes may be followed in Table I in patients H S and J J where the stimulus was apparently severe infection, and in patients C. C., J J, and A. F., when serum protein was regenerating rapidly during convalescence. In

patients C. C., J J and A. F. in whom the serum proteins regained the normal value following diuresis, the globulin values obtained by the precipitin method eventually became normal. The failure to demonstrate significant amounts of altered globulin in patient J J and others during the edematous stage may be the result of such forces. No correlation could be demonstrated between any single globulin sub-fraction and the excessive value obtained by the precipitin method. The difficulties involved in the separation of the glo-

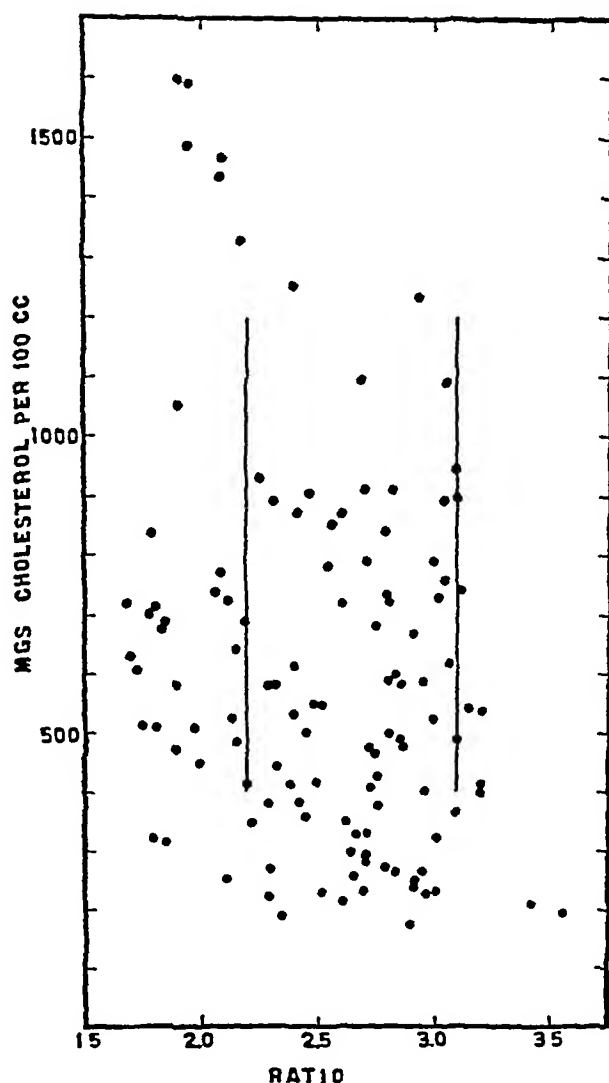


FIG. 4 RELATION OF SERUM CHOLESTEROL TO THE RATIO OF CHOLESTEROL ESTER TO FREE CHOLESTEROL

The lines indicate the limits of the ratio for normal sera.

bulin sub-fractions by salt precipitation in highly lipoidal serum are legion. Satisfactory separation could not be obtained in this laboratory even with the use of a high-speed centrifuge. The data obtained on sera subjected to fractional analysis, presented in Table II, show that the high precipitin values cannot be related to a change in any one globulin sub-fraction.

3 Can an arbitrary level of altered protein be selected which will distinguish nephrotic from nephritic patients in order to secure more accurate diagnosis and prognosis in kidney disease in children? Seventeen cases of acute glomerular ne-

phritis were studied in a similar fashion. In 11 cases prolonged studies were possible and in the remaining 6 cases one or two observations only were made. The data from a few patients are collected in Table III. In all cases the altered albumin was less than 50 per cent of the total albumin by Howe's method, however, the total amount of altered albumin was often as much as that found in some nephrotic patients. Change in the globulin fraction was rarely present and was always minimal. The contrast between the findings in acute glomerular nephritis and in nephrosis is brought out in Figure 5, in which the highest percentage of altered albumin found in each patient is charted. Three nephrotic sera and 3 nephritic sera presented between 40 and 50 per cent of altered albumin. In 2 of the nephrotic sera presenting such changes an accompanying change in the globulin fraction of 40 per cent or more appeared at some time during the course of the disease. The altered albumin in acute glomerular nephritis is always transient, while in nephrosis the change usually persists for long periods of time. In the 2 cases of nephritis in which the percentage of altered albumin was above 40 per cent on admission, *i.e.*, in patients J. D. and R. V., the percentage of altered albumin decreased markedly within 3-weeks' time.

Thus an arbitrary level of 40 per cent of altered protein in the albumin fraction will serve to differentiate between most cases of acute glomerular nephritis and nephrosis, while a value of 50 per cent or more has been found only in nephrosis. A significant amount of altered albumin accompanied by a significant amount of altered globulin seems to indicate nephrosis as does a persistence of a high percentage of altered albumin for a month or longer.

It was hoped that the precipitin test would be of aid in the differential diagnosis between chronic glomerular nephritis with the nephrotic syndrome and pure nephrosis. Four patients with chronic glomerular nephritis were available for study. The data are assembled in Table IV. In 2 of these patients, M. W. and A. A., vascular damage was prominent and nephrotic symptoms were minimal. Edema was not marked, the serum albumin levels were only moderately low, and the serum cholesterol level was only slightly elevated.

TABLE II

Relationship of the various globulin fractions to the amount of globulin determined by the precipitin method

Patient and disease	Date	Howe's method					Precipitin	Comment
		Albumin	Englobulin	Pseudo-globulin I	Pseudo-globulin II	Total globulin		
		grams per 100 cc.					grams per 100 cc.	
S. S. Nephrosis	May 27 1937	1.41	0.25	1.07	1.12	2.44	2.00	Edematous
	September 22 1937	1.19	1.52	0.00	0.76	2.28	2.41	Edematous
	February 21 1938	1.38	0.06	1.14	0.96	2.16	3.33	Edematous
	May 26 1938	1.81	0.28	1.33	0.87	2.48	3.57	Edematous
C. C. Nephrosis	April 24 1936	1.68	0.18	1.25	1.05	2.48	3.02	Edematous
	April 30 1936	2.57	0.15	1.48	1.07	2.70	1.55	Diuresis in progress
	May 13 1936	3.51	0.14	0.82	1.10	2.06	1.94	Regeneration of serum protein
	June 3, 1936	3.44	0.09	0.68	1.44	2.21	2.05	
	June 17 1936	3.11	0.09	1.34	0.96	2.39	1.55	
	October 3 1936	1.95	0.26	1.21	1.00	2.47	3.75	Edematous
	October 13 1936	2.47	0.17	1.57	0.69	2.43	3.32	Diuresis in progress
	February 20 1937	4.42	0.17	1.26	1.11	2.54	2.76	Regeneration of serum protein
	November 3 1937	4.06	0.11	0.54	1.04	1.69	1.62	
A. F. Nephrosis	February 10 1936	0.85	0.16	1.17	0.81	2.14	0.80	Edematous
	February 19 1936	1.72	0.31	1.15	1.14	2.60	4.30	Diuresis in progress
	March 5 1936	2.55	0.43	1.14	1.15	2.72	4.67	Regeneration of serum protein
	March 11, 1936	3.08	0.36	1.45	1.03	2.84	3.59	
	April 8 1936	3.41	0.39	0.82	1.31	2.52	2.30	
	May 6, 1936	3.76	0.53	0.79	1.10	2.42	2.31	
	July 14, 1936	4.26	0.54	0.90	1.06	2.50	2.35	
	September 22 1937	5.23	0.04	1.22	0.87	2.13	2.70	
J. J. Nephrosis	November 15 1937	1.67	0.68	1.27	0.22	2.17	2.84	Edematous. Bronchopneumonia
	November 26 1937	2.56	0.49	1.46	0.79	2.74	5.37	Regeneration of serum protein
	December 13 1937	4.14	0.14	1.43	0.94	2.51	5.00	
	September 9 1938	5.00				1.86	1.97	
M. T. Eczema. Nutritional edema	April 23 1936	1.15	0.18	0.58	0.62	1.38	2.02	Regeneration of serum protein
	May 4 1936	2.01	0.85	1.39	1.23	3.47	5.10	
	May 21, 1936	3.71	1.52	1.45	0.92	3.89	8.72	
J. S. Ulcerative colitis. Malnutrition	May 18 1936	2.52	0.64	1.60	0.67	2.91	5.38	Regeneration of serum protein
	June 1 1936	3.69	0.90	1.69	1.15	3.74	7.40	
	June 24 1936	3.74	1.06	0.62	1.36	3.55	6.51	
P. S. Eczema. Nutritional edema	April 14 1936	1.56	0.00	0.68	0.63	1.31	1.70	Regeneration of serum protein
	April 25 1936	2.61	0.17	0.93	1.23	2.33	2.18	
	May 12 1936	4.56	0.14	1.02	1.15	2.31	2.66	

The serological changes of the serum proteins were likewise not marked and there was little change in the globulin fraction. Both children died in uremia. In contrast in the other 2 patients, E. L. and E. P., the nephrotic manifestations were marked, generalized anasarca was present, the serum albumin levels were low and the serum cholesterol was high. In these 2 patients the serological changes were marked in both the albumin and the globulin fractions.

These patients died of pneumococcal septicemia and peritonitis. Thus it is not possible to differentiate between chronic glomerular nephritis (nephrotic stage) and pure nephrosis by the precipitin method.

4. What is the nature of the altered protein? Previously it had been determined that the failure of nephrotic serum to react completely with specific antisera was not due to the high cholesterol content nor to the distribution of the globulin

TABLE III

*Analyses of sera from cases of acute glomerular nephritis
Comparison of Howe's method and the precipitin method*

Patient, age, date	Albumin			Globulin			Al- bumin- uria	Ede- ma	Cho- les- terol
	Howe	Precip- itin nor- mal	Al- tered	Howe	Precip- itin nor- mal	Al- tered			
	grams per 100 cc.								mgs per 100 cc.
E P 8 years									
March 11 1937	4.08	3.28	0.82	2.71	2.78	0	++	0	217
March 25 1937	3.84	2.94	0.90	2.49	2.21	0.28	+++	0	331
April 9, 1937	3.74	3.19	0.55	2.12	1.95	0.17	++	0	303
April 27, 1937	3.88	3.19	0.67	1.95	1.60	0.36	++	0	284
May 18, 1937	4.41	3.72	0.69	1.92	1.55	0.27	++	0	306
June 1, 1937	4.46	3.52	0.94	1.84	1.76	0.08	++	0	307
June 11, 1937	4.52	4.05	0.47	2.09	2.13	0	++	0	305
September 22, 1937	4.64	4.19	0.45	2.11	2.17	0	+	0	
R. DeG 11 years									
February 11 1936	3.35	3.25	0.09	2.42	2.40	0.02	+++	++	155
February 18, 1936	4.20	3.90	0.30	3.49	3.95	0	++	±	213
March 5 1936	4.17	4.18	0	3.20	3.20	0	±	0	216
March 25, 1936	4.44	3.83	0.61	2.77	2.88	0	±	0	
April 1 1936	4.20	4.10	0.10	3.20	3.57	0	+	0	
April 15 1936	4.21	4.11	0.10	2.73	2.92	0	±	0	162
April 29 1936	4.75	4.97	0	2.62	3.12	0	±	0	162
May 13, 1936	4.92	5.01	0	2.30	2.94	0	±	0	168
July 1, 1936	4.68	4.90	0	2.30	2.52	0	±	0	177
October 23, 1936	5.38	4.95	0.43	2.57	3.05	0	0	0	161
December 9, 1936	4.72	4.51	0.21	2.04	2.80	0	0	0	156
January 13, 1937	4.49	4.43	0.06	2.06	2.07	0	0	0	167
L. McM 7 years									
April 7 1936	2.95	2.63	0.32	2.80	2.94	0	+++	+	210
April 23, 1936	3.72	3.06	0.66	3.02	2.75	0.27	+	0	283
May 19 1936	4.37	3.71	0.66	2.73	3.12	0	+	0	
July 1 1936	4.03	3.99	0.04	2.32	2.33	0	+	0	154
October 23, 1936	4.71	4.66	0.05	2.54	2.78	0	0	0	
J. McG 9 years									
November 3 1936	3.75	3.48	0.27	2.45	2.44	0	+	++	206
November 10 1936	4.49	3.96	0.53	2.56	2.79	0	+	0	
November 17 1936	4.63	3.99	0.64	2.64	2.50	0.14	+	0	256
November 30 1936	4.60	3.97	0.63	2.64	2.49	0.15	+	0	281
December 6, 1936	4.64	4.16	0.48	2.32	2.37	0	±	0	
December 16, 1936	4.46	4.34	0.12	2.57	1.88	0.69	±	0	242
February 10 1937	4.53	4.50	0.03	2.03	2.00	0.03	0	0	214
April 21, 1937	4.85	4.13	0.72	1.69	1.75	0	0	0	205
J. D 5 years									
February 3, 1937	2.93	1.50	1.43	3.06	3.06	0	0	++	193
February 11 1937	3.64	1.93	1.65	3.16	3.24	0	0	±	277
February 23, 1937	4.37	3.66	0.71	2.68	2.48	0.20	0	0	284
March 25, 1937	4.65	3.94	0.71	2.23	2.33	0	0	±	209
April 10, 1937	4.64	4.58	0.06	2.24	1.85	0.39	0	0	240
April 16, 1937	4.50	4.53	0.27	2.02	1.83	0.14	0	0	209
May 5, 1937	4.45	3.57	0.88	2.26	2.00	0.26	0	0	205
December 16 1937	4.80	4.32	0.58	1.71	1.84	0	0	0	183
R. V 10 months									
February 2, 1937	3.12	1.81	1.31	2.79	2.67	0	±	±	97
February 10 1937	3.33	2.11	1.22	3.52	4.07	0	±	0	97
March 3, 1937	4.02	2.83	1.14	2.99	3.07	0	±	0	181
March 20 1937	3.95	2.96	1.02	3.74	4.05	0	±	0	204

sub-fractions. It was assumed that the failure resulted from the presence of varying amounts of an altered albumin and an altered globulin, which were measured by Howe's method but which were inactive immunologically to normal antisera. Support for this assumption has been secured in experiments with absorbed antisera. If nephrotic albumin is a mixture of normal albumin and altered albumin, and, similarly, if ne-

phrotic globulin is composed of normal and altered globulin, an antiserum prepared against such nephrotic protein should contain antibodies both to the normal and the altered protein on one condition, namely, that the altered protein be capable of stimulating antibody production in the rabbit. If the antibodies to normal serum were absorbed out, the resulting absorbed antisera should contain antibodies to the altered protein only. The absorbed antisera should then become valuable tools for the identification of altered protein in various pathological sera. If an altered protein could be demonstrated in nephrotic sera by means of the absorbed antisera, the experiments would support the conclusion that the serum proteins in nephrosis differ from normal serum proteins.

Preparation of the antisera. Samples of blood serum were accumulated over a period of 3 weeks from patient H S at a time when precipitin analyses indicated that about 60 per cent of the albumin was abnormal. From the pooled samples the albumin was isolated as previously described (2) and injected into rabbits to produce an antinephrotic albumin serum. The antibodies to normal albumin were then absorbed out by the addition of small increments of normal serum albumin. The precipitate was always removed by centrifugation with decantation of the supernatant before more albumin was added, and the process was continued until no further precipitate was thrown down. Theoretically, the absorbed antiserum now contained antibodies to the altered albumin only.

Similarly, 3 antinephrotic globulin sera were prepared from nephrotic globulin isolated from patients B G, H S, and J F. In these patients precipitin analyses at the time the blood was withdrawn indicated that about 85 per cent, 70 per cent, and 50 per cent, respectively, of the globulin was abnormal. The antibodies to normal globulin were then absorbed out by the successive addition of small amounts of normal globulin. After a considerable excess of normal globulin had been added, a trace of precipitate continued to form. It was felt that this trace represented the interaction with one of the weaker antigenic components, and that it could be safely ignored, since the antisera were always checked for cross precipitation with normal globulin before use.

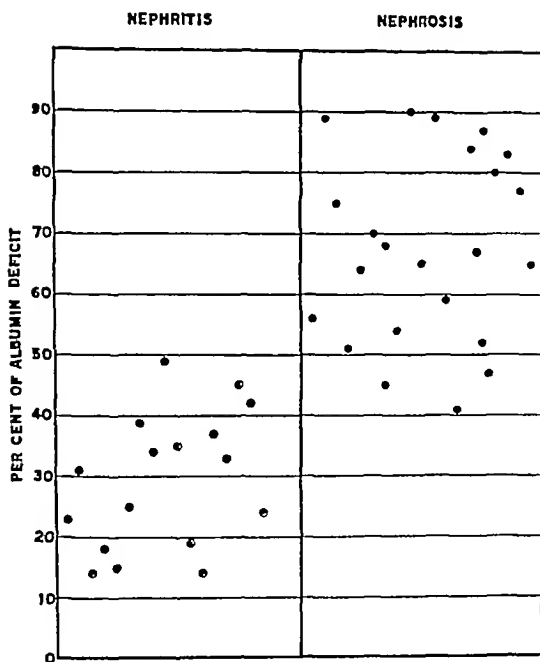


FIG. 5 COMPARISON OF THE MAXIMAL QUANTITIES OF ALTERED ALBUMIN IN NEPHRITIC AND NEPHROTIC PATIENTS

Thus several types of antisera were available for study

- 1 Anti normal albumin serum
- 2 Unabsorbed antinephrotic albumin serum theoretically containing antibodies to normal albumin and altered albumin
- 3 Absorbed antinephrotic albumin serum theoretically containing antibodies to the altered albumin only
- 4 Anti normal globulin serum.
- 5 Unabsorbed antinephrotic globulin serum which should contain antibodies to normal globulin and to altered globulin.
- 6 Absorbed antinephrotic globulin serum which should contain antibodies to the altered globulin only

Tests with the various anti-albumin sera In order to study the reactions of normal serum al-

bumin and nephrotic serum albumin both substances were set up against the 3 antisera the anti normal albumin serum the unabsorbed anti nephrotic albumin serum, and the absorbed anti nephrotic albumin serum. All tests were performed with approximately 0.2 mgm. of antigen protein and 1 cc. of antiserum in a total volume of 3 cc., that is, within the optimum range of the antigen antibody reaction. To guard against error the supernatants were always tested for excess antibody. The antigen antibody precipitates formed were centrifuged, washed twice with saline and the nitrogen content determined by the micro-Kjeldahl method as previously described (2). The normal albumin used in the tests had been isolated from pooled human serum by precipitation of the globulin at half saturation with ammonium sulphate and subsequent dialyzation of the filtrate against tap water until free from sul-

TABLE IV

Analyses of sera from cases of chronic glomerular nephritis Comparison of Howe's method and the precipitin method

Patient, age date	Albumin			Globulin			Albuminuria	Edema	Cholesterol	Comment
	Howe	Precipitin normal	Altered	Howe	Precipitin normal	Altered				
	grams per 100 cc								mgm per 100 cc	
E L May 20, 1936	1 17	0 31	0 86	3 02	1 56	1 46	++++	++++	1117	Edema present 6 months May 24, 1936 Septicemia Peritonitis Pneumococcus Died
E P May 5, 1937	2 26	1 48	0 78	1 78	1 01	0 77	++++	0	666	Edema present 4 months After diuresis
June 28, 1937	1 30	0 26	1 04	1 38	0 77	0 61	++++	++++	281	Septicemia Peritonitis Pneumococcus Died June 29, 1937
M W November 22, 1934	2 55	1 94	0 61	1 65	1 10	0 55	+++	+	475	Edema present 3 months
March 7, 1935	3 22	2 81	0 41	2 85	3 07	0		—	270	Convalescence scarlet fever
March 14, 1935	3 27	2 80	0 47	2 61	2 78	0	++	0	310	
April 10, 1936	1 74	0 89	0 85	1 78	2 56	0	++++	0	234	?Peritonitis Died January 21, 1938 Uremia
A. A February 6, 1937	2 51	1 25	1 26	2 11	2 24	0	++++	++	193	Edema present 6 months Sinusitis Tetany
September 15, 1937	4 19	3 48	0 71	2 27	2 41	0	+++	++	282	Hemiplegia Died December 1, 1937 Uremia

phate ion The nephrotic albumin was that which had served in the preparation of the antinephrotic albumin serum Only a few tests could be carried out with the nephrotic albumin because the quantity of material was limited, and therefore a saline dilution of serum from the same patient was substituted in most of the tests The sera of the remaining nephrotic patients were likewise merely diluted with saline without further manipulation The results obtained are depicted in Table V

It is evident from the table that normal albumin gave an adequate precipitate with its homologous antiserum as well as a fairly good precipitate with the unabsorbed antinephrotic albumin serum, which indicates that the unabsorbed antinephrotic albumin serum contains antibodies in moderate quantity to normal albumin However, no precipitate was obtained with the absorbed antinephrotic albumin serum, which proves that all of the antibodies to normal albumin were completely removed from the absorbed antiserum Now when nephrotic albumin was set up against the

anti-normal albumin serum a precipitate was thrown down which was less than one would expect if all of the protein were normal albumin If the nephrotic albumin from the various patients with nephrosis was composed entirely of normal albumin a precipitate of about 300 mgm.

TABLE V

Reactions of normal and nephrotic albumin with various anti-albumin sera

Albumin	Sample	Albumin antisera Nitrogen in precipitate		
		Normal	Nephrotic unabsorbed*	Nephrotic absorbed*
mgm		mgm	mgm	mgm
221 Normal	Isolated H S *	306	204	0
150 Nephrotic	Isolated H S *			0
130 Nephrotic	H S *	132	114	0
200 Nephrotic	S S	088	065	010
200 Nephrotic	W S	111		0
180 Nephrotic	G D	034		0

* Homologous with unabsorbed and absorbed antinephrotic albumin sera

of nitrogen would be expected, while actually the values obtained were always less. When nephrotic albumin reacted with the unabsorbed antinephrotic albumin serum a moderate precipitate was formed but with the absorbed antinephrotic albumin serum there was no precipitate except in 1 case when a trace was obtained. These results may be interpreted to indicate that the altered protein in the albumin fraction is not capable of stimulating precipitin antibodies in the rabbit. The experiments need confirmation by the preparation of antisera to the nephrotic albumin isolated from other patients.

preparation of 1 of the antisera was set up with the anti normal globulin serum and yielded a moderate precipitate with the homologous unabsorbed antinephrotic serum a larger precipitate was formed. These precipitates would be expected if nephrotic globulin was a mixture of normal and altered protein. Now when isolated nephrotic globulin was set up against the 3 absorbed antinephrotic globulin sera significant precipitates were formed in each instance, irrespective of whether the homologous antigen had served in the preparation of the antisera. Hence, nephrotic globulin isolated from 3 patients contained a sub-

TABLE VI
Reactions of normal and nephrotic globulin with various anti-globulin sera

Globulin	Sample	Globulin antisera. Nitrogen in precipitate				
		Normal	Nephrotic* unabsorbed	Nephrotic* absorbed No. 1	Nephrotic absorbed No. 2	Nephrotic ab- sorbed No. 3
<i>mgm</i>		<i>mgm</i>	<i>mgm</i>	<i>mgm</i>	<i>mgm</i>	<i>mgm</i>
.236 Normal	Isolated	204	083	031	019	010
.243 Nephrotic	B G * Isolated	108	.215	146	092	086
.201 Nephrotic	S S Edematous April 22	149	150	125		
183 Nephrotic	S S Edematous May 27	146		108	093	091
.228 Nephrotic	S S Edematous September 22	186	159	118	084	072
156 Nephrotic	A. B Edematous	166	129	097		
185 Nephrotic	G D Edematous	099		082		
173 Nephrotic	J J Edematous	144		082		
189 Nephrotic	W S Edematous	162		090		
191 Normal	D L Edema free†	151		.051		
170 Normal	A. F Edema free†	175	096	039	030	021

* Homologous with unabsorbed and absorbed antinephrotic globulin sera (No. 1)

† Cured nephrotic patients.

Tests with the various anti-globulin sera. Similarly in order to compare normal globulin and nephrotic globulin both substances were set up against the various anti-globulin sera the anti normal globulin serum the unabsorbed antinephrotic globulin serum and the 3 absorbed antinephrotic sera. The assembled data in Table VI reveal marked deviation from the analogous albumin studies in some respects. Normal globulin reacted with its homologous antiserum with the unabsorbed antinephrotic globulin serum a moderate

stance which is not found in normal globulin and which stimulated precipitin antibody formation in the rabbit.

Sera from nephrotic patients were now diluted with saline without further manipulation and set up against the various anti-globulin sera, the results are included in Table VI. The results were analogous to those obtained with the isolated nephrotic globulin in all patients with generalized anasarca i.e. precipitates of about the same magnitude were formed with the absorbed antine-

normal globulin This finding was predictable as an examination of Table I shows that the serum of patient A F showed no altered globulin

Is altered protein present in the sera of patients with diseases other than nephrosis and nephritis? Previous studies of a few patients with edema from other causes than nephritis or nephrosis and a number of dogs with experimental nutritional

edema had failed to disclose significant amounts of altered protein in the sera These studies have now been extended to include patients exhibiting any one of the disturbances which characterize the nephrotic syndrome Two eczematous patients with nutritional edema resulting from insufficient ingestion of protein were included as examples of severe malnutrition in allergic indi-

TABLE VII

Analyses of sera from cases of malnutrition, hypothyroidism, or hepatomegaly Comparison of Howe's method and the precipitin method

Patient age	Disease	Date	Albumin			Globulin					
			Howe	Precip- itin normal	Al- tered	Total			Fractions		
						Howe	Precip- itin normal	Altered	Euglob- ulin	Pseudo- globulin I	Pseudo- globulin II
			grams per 100 cc			grams per 100 cc					
R H 6 years	Laennec's cirrhosis	March 8, 1937	2 87	2 28	0 59	2 27	2 34	0	0 32	1 00	0 95
		April 15, 1937	2 27	1 98	0 29	1 56	1 61	0	0 03	0 54	0 99
		April 19, 1937	2 42	1 88	0 54	1 39	1 32	0 07			
R T 11 years	Laennec's cirrhosis	March 26, 1936	3 90	3 58	0 32	4 20	8 03	0	0 85	1 84	1 51
L M 9 years	Hepatitis	March 8, 1937	3 45	3 51	0	5 53	8 13	0	1 77	2 79	0 97
W H 16 months	? Cirrhosis	September 14, 1937	5 31	4 71	0 60	1 90	1 12	0 78	0	0 59	1 31
C E 6 years	? Liver abscess	November 20, 1936	3 74	3 07	0 67	5 47	11 34	0	2 19	2 36	0 92
W L 11 years	Amyloidosis	April 20, 1937	2 78	1 78	1 00	3 03	5 41	0	0 65	1 09	1 29
N A 5 years	? Banti's disease	December 4, 1937	5 07	4 84	0 23	2 29	2 60	0	0 30	0 89	1 10
E J 4 years	Aplastic anemia	December 4, 1936	3 16	3 14	0 02	3 41	7 00	0	0 82	1 86	0 73
J R 2 months	Syphilitic hepatitis	March 22, 1937	3 50	3 20	0 30	3 27	3 22	0 05			
J G 20 months	Hypothy- roidism	February 5, 1936	5 08	4 37	0 71	1 50	0 99	0 51			
M T 6 months	Nutritional edema	April 23, 1936	1 15	0 73	0 42	1 38	2 02	0	0 18	0 58	0 62
		May 4, 1936	2 01	0 96	1 05	3 47	5 10	0	0 85	1 39	1 23
		June 1, 1936	3 71	2 60	1 11	3 89	8 72	0	1 52	1 45	0 92
T D 4 months	Nutritional edema	March 19, 1937	1 85	1 66	0 19	1 43	0 95	0 48			
		April 5, 1937	2 75	2 78	0	1 68	0 89	0 79			
P S 2 years	Nutritional edema	April 14, 1936	1 56	1 09	0 47	1 31	1 70	0	0	0 68	0 63
		April 25, 1936	2 61	2 07	0 54	2 33	2 18	0 15	0 17	0 93	1 23
		May 12, 1936	4 56	3 87	0 69	2 31	2 66	0	0 14	1 02	1 15
J S 12 years	Ulcerative colitis Nutritional edema	May 18, 1936	2 52	1 61	0 91	2 91	5 38	0	0 64	1 60	0 67
		June 1, 1936	3 69	3 15	0 54	3 74	7 40	0	0 90	1 69	1 15
		June 24, 1936	3 74	3 80	0	3 55	6 51	0	0 62	1 36	1 57

viduals. One patient with ulcerative colitis exhibited hypoproteinemia because of failure to absorb ingested protein. One patient with hypothyroidism was selected because of the high cholesteremia. Since the liver is supposed to share in the synthesis of serum protein, a number of patients with various types of severe liver damage were investigated. The data are assembled in Table VII.

In none of the patients were maximal amounts of altered protein encountered, nevertheless in the 2 patients with nutritional edema due to an extreme degree of hypoproteinemia, the values found were at the lower level of the empirical borderline selected to differentiate nephrotic sera. In 1 of these patients M. T. with a total protein of 2.53 grams per 100 cc. of serum, there was 48 per cent of altered albumin. In a similar patient, T. D., with a total serum protein of 3.58 grams per 100 cc., the albumin was normal but the globulin revealed 47 per cent of altered protein.

In no case of severe liver damage analyzed was there a significant amount of altered albumin or altered globulin. In 1 patient W. L., the altered albumin was found to be 36 per cent, this child was in the terminal stage of tuberculous enteritis with severe malnutrition and amyloidosis subsequently proven by autopsy. The globulin values obtained by the precipitin method in the cases of liver damage were usually high since the euglobulin and pseudoglobulin fractions were also high (1), and in no case was there an appreciable amount of altered globulin.

Sera from most of the patients with liver damage were tested against the 3 absorbed antinephrotic globulin sera to determine whether the globulin from these patients contained any of the altered protein found in nephrosis. No reaction occurred and the conclusion seems justified that in the patients with liver damage the serum globulin is not identical with that found in nephrosis. Further studies are necessary before an interpretation of the results in terms of the relation of altered protein to the etiology of nephrosis can be hazarded. It would seem however that even in extremely severe states of malnutrition repre-

sented by such patients as M. T., T. D., and J. S., without liver disease, and R. H. and W. L. with liver damage changes in the serum protein never reach the maximal values so commonly found in nephrosis.

CONCLUSIONS

In the sera of 23 cases of nephrosis examined during the edematous stage the quantitative precipitin method revealed evidence of an altered protein in the albumin fraction which was usually associated with an altered protein in the globulin fraction. Maximal amounts of altered protein may be present very early in the course of the disease. The altered protein persists for long periods of time.

The patients whose sera show maximal amounts of altered protein are not more susceptible to superimposed infection than those with lesser change; no relation could be demonstrated between the amount of altered serum albumin and the albuminuria, cholesteremia, or edema.

Quantitative precipitin studies on the sera of 17 cases of acute glomerular nephritis revealed minimal amounts of altered protein in the albumin fraction with rarely any associated altered globulin. The changes were transient.

Evidence has been presented in support of the assumption that the failure of the protein of nephrotic serum to react completely with rabbit antisera is due to the presence of altered protein in the albumin and globulin fractions. The altered protein in the globulin fraction of 3 patients forms antibodies when injected into the rabbit which do not react with normal globulin; the antibodies react with nephrotic sera drawn during the edematous stage of nephrosis but not with the sera of cured nephrotics. The altered protein in the serum albumin of 1 patient was not capable of stimulating antibody formation in the rabbit.

No evidence was obtained from precipitin studies on the sera of patients with various diseases other than nephrosis to indicate that severe malnutrition, hypothyroidism or liver damage causes serological changes of the same magnitude as those found in nephrotic patients.

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NATURE OF PERIPHERAL RESISTANCE IN ARTERIAL HYPERTENSION

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The nature of the arteriolar resistance and its distribution in the body remain the fundamental problem in the study of arterial hypertension in man. Since the cardiac output is normal in this state (1), the "average arteriolar resistance" at rest must be increased. The extent to which the peripheral resistance is increased in various tissues can be determined by comparing the blood flow in the different tissues in both normal and hypertensive subjects under similar conditions. If in arterial hypertension the peripheral resistance in any one part is not increased the blood flow in that part will be greater than in normal subjects.

Since the cardiac output at rest is normal in arterial hypertension, the "average tissue blood flow" must also be normal. This has been confirmed by the demonstration that in hypertensive subjects the blood flow is normal in the hand and forearm (2, 3). While it is of interest to gather further information on the 'resting' blood flow in other organs in normal and hypertensive subjects, it is of particular importance to ascertain whether the increased peripheral resistance in arterial hypertension can be lowered to a normal level in any one organ by potent vasodilating stimuli. If the increased peripheral resistance which exists at rest in arterial hypertension can be lowered to normal in any one organ as manifested by a faster blood flow than is present in that organ in normal subjects under similar conditions, then the increased peripheral resistance at rest is the result of functional vasoconstriction, and is not the result of permanent irreversible structural changes in the vessels. If the increased peripheral resistance present in arterial hypertension, however, cannot be reduced to normal by powerful vasodilating stimuli, as indicated by the finding of the same blood flow in the tissues of both normal and hypertensive subjects when the vessels are maximally dilated then there are at least two obvious explanations. The chemical or other factors, which cause the vasoconstriction

and the increased peripheral resistance, remain active in the presence of the various physiological stimuli applied and prevent the vessels from dilating as widely as normally, or the increased peripheral resistance is the result of permanent structural changes and therefore, the vessels cannot dilate as widely as normally.

Other investigators have shown that the increased peripheral resistance, which is present in the usual types of clinical arterial hypertension, is not reflex or neurogenic in origin. Prinzmetal and Wilson (2) demonstrated that on eliminating the vasoconstrictor influences by heating the body, or by novocainization of the sympathetic ganglia, the blood flow in the forearm in subjects with arterial hypertension increased as much as, but no more than in normal subjects. They concluded therefore, that, as the peripheral resistance remained increased in arterial hypertension after the removal of all vasoconstrictor impulses, it could not be neurogenic in origin. Recent work however has shown that the method used by Prinzmetal and Wilson for determining the blood flow in the forearm measured not only the arterial inflow in the forearm, but also a considerable portion of the venous blood returning from the hand (4, 5).

Using a calorimeter, Pickering (3) measured in both normal and hypertensive subjects the blood flow in the hand after the elimination of vasoconstrictor impulses by heating the body. This procedure produced the same increase in blood flow in both the normal and hypertensive subjects. He concluded, therefore that the increased peripheral resistance in arterial hypertension was not neurogenic in origin. These investigators (2, 3) likewise observed that the increase in blood flow in the forearm after arterial occlusion was the same in normal and hypertensive subjects. Both of the above studies (2, 3) clearly showed that, in the usual clinical types of hypertension the peripheral resistance in the hand and forearm was increased at rest and that

while the vessels were capable of dilating in response to the stimuli employed, they never dilated to as great an extent as did normal vessels under similar circumstances. It was possible, however, that the high peripheral resistance present in the skin in arterial hypertension had not been reduced to normal because the stimuli employed had not produced maximal dilatation. Observations of the blood flow in the hand and foot (6) have shown that heating the body was usually not as effective a stimulus for inducing complete vasodilatation as was the combination of local heat to the skin and heating the body, which was produced by placing the parts to be studied in a water bath at 43° C.

The present investigation has been carried out to determine with reliable methods whether the high average arteriolar resistance, which is present throughout the body at rest in arterial hypertension, can be reduced to normal in any one tissue by the use of appropriate local vasodilating agents. The skin of the hand and foot, the muscles of the forearm, and the brain were the tissues selected for study. In order to determine to what extent permanent structural changes in the vessels entered into the production of the increased peripheral resistance in arterial hypertension, the peripheral blood flow was studied in 2 hypertensive subjects both before and after the high arterial pressure had been greatly reduced by malaria. These 2 subjects also offered a good opportunity for studying the vasomotor reflexes in the same persons at two widely different levels of arterial pressure.

METHODS

Since a given vasodilating stimulus is not uniformly effective in all parts of the body (5), different means were used for producing vasodilatation in the skin, muscle, and brain.

Vasodilatation was obtained in the hand and foot by maintaining the water baths in plethysmographs at a temperature of 43° C. As two parts (both feet, or hand and foot) were usually studied at one time, the subjects became hot enough to sweat, and the vasodilating effect of the local heat was supplemented by the effect of heating the body as a whole. The blood flow in the hand and foot was measured by plethysmographic methods (7, 8) and recorded as cubic centimeters per minute per 100 cc. of tissue. As previously stated (6), when the vessels are widely dilated, the inflow curves are much more accurate in the foot than in the hand. This is the

result of the slower blood flow and the relatively greater venous bed in the foot, and of the higher hydrostatic pressure in the foot plethysmograph. Each determination consisted of an average of five separate tracings, and three or more separate determinations were made at 5-minute intervals until the blood flow became constant. The subjects rested on a table in the horizontal position with the water in the plethysmographs at a temperature of 43° C for at least 30 minutes before readings were taken. The room temperature was not carefully controlled because when the parts studied were immersed in water at 43° C. ordinary variations in room temperature did not appreciably influence the blood flow.

The vessels of the muscles of the forearm were dilated by a combination of exercise and arterial occlusion. The blood flow in the forearm was measured in the plethysmograph described by Lewis and Grant (9), and recorded as cubic centimeters per minute per 100 cc. of tissue. A cuff distal to the plethysmograph was inflated to 300 mm Hg before the blood flow was measured in the forearm. The subjects compressed a firm rubber bulb every 5 seconds for 3 minutes. During the period of exercise blood was prevented from entering the forearm by inflating a cuff on the upper arm to 300 mm Hg. After the exercise had been completed the proximal occluding cuff was rapidly deflated, and the first rapid inflow of blood into the forearm was measured. It was essential to measure the first inflow of blood, because after a few seconds the veins emptying the forearm became so engorged that the application of the "collecting cuff" produced a fluid wave large enough to distort the inflow curves.

The vessels of the brain were dilated by progressively decreasing the blood flow to the brain by placing the subjects in the upright position after the oral ingestion of 0.18 gram (3 grains) of sodium nitrite (10). Syncope was induced in both normal and hypertensive subjects, and the level of the arterial pressure at which syncope occurred was recorded. The occurrence of syncope indicated that the blood flow through the brain had fallen to approximately the same level in both hypertensive and normal subjects. Therefore, the arterial pressure at that time indicated whether comparable vasodilatation had taken place in the brains of the two groups.

Two subjects with arterial hypertension and syphilis were given malaria. In both instances a marked, temporary fall in arterial pressure occurred. The blood flow in the foot at 43° C. and the vasomotor reactions in the foot were measured before and after the fall in arterial pressure. In 1 subject the blood flow in the forearm after exercise was measured before and after the decrease in arterial pressure.

RESULTS

Subjects investigated. Sixteen patients with arterial hypertension were studied. The average age of this group was 43 years. The average systolic pressure was 206 mm Hg, the highest 270 mm and the lowest 162 mm, the average dias-

tolic pressure was 131 mm, the highest 162 mm. and the lowest 110 mm. Clinically, the patients were divided as follows: 3 cases of malignant hypertension following pyelonephritis, 5 cases of malignant hypertension of unknown etiology, 8 cases of essential hypertension. As all the cases did not have complete genito-urinary studies, some cases of hypertension from pyelonephritis may have been overlooked. No manifestations of failure of the heart were present at the time of examination. There were no cases of acute glomerular nephritis or, as far as could be determined, of chronic glomerular nephritis. Pulsations in all the peripheral vessels were easily palpable with the exception of 1 case of essential hypertension in whom the posterior tibial pulse in the left foot was not felt.

Blood flow in the skin. The blood flow in the foot with the vessels dilated by local heat of 43° C averaged 217 cc per minute per 100 cc. of foot in 29 feet from 16 subjects with arterial hypertension. The greatest flow was 34 cc. and the lowest 14 cc. In a previous study (6), the average blood flow in 48 feet from a group of 34 normal subjects ranging in age from 17 to 67 years was 171 cc. per minute per 100 cc. of foot. The average foot flow in the hypertensive group was 27 per cent faster than in the normal group. The normal subjects however, had a wide age distribution, while 14 out of the 16 hypertensive subjects were between 30 and 50 years of age. When the values for the blood flow, therefore are broken down into corresponding decades as in Figure 1 it is clear that the blood flow in the foot, with the vessels dilated by heat, is not significantly less in normal than in hypertensive subjects. In these patients there is no apparent correlation between the etiology of the hypertension and the blood flow in the foot. In 4 of these hypertensive subjects the average blood flow in the hand at 43° C was 31 cc. per minute per 100 cc. of tissue. In 18 normal subjects (6) the average hand flow at 43° C was 32 cc. Again the blood flow in the dilated hand is not significantly different in normal and in hypertensive subjects.

The simultaneous determination of the blood flow in the hand and foot at 43° C in these cases of arterial hypertension demonstrates the fallacy of drawing conclusions about the general state

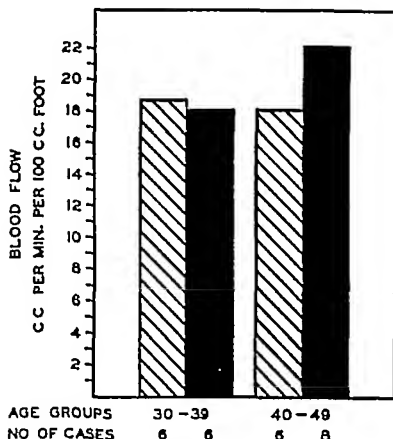


FIG. 1 THE BLOOD FLOW IN THE FOOT AT 43° C. IN NORMAL AND IN HYPERTENSIVE SUBJECTS

Barred columns represent normal, solid represent hypertensive subjects. There was no significant difference in the blood flow in the foot in the two groups.

of the peripheral resistance from studies of the blood flow in one part of the body. Thus the foot flow at 43° C in 1 subject E. P. averaged 34 cc. This value was very high but the blood flow in the hand at 43° C and the blood flow through the brain as estimated by the oxygen difference between arterial and internal jugular blood were normal.

Blood flow in the muscle. The blood flow in the muscles of the forearm after a standard amount of exercise was measured in 6 normal and 3 hypertensive subjects. While the standardization of the exercise was not exact it did not seem advisable to make it more precise, since the variations in amount of muscle in the forearm and in the general physical training of the subjects were factors beyond control. During the period of exercise blood was excluded from the forearm in order to secure maximal dilatation of the muscle vessels in as short a time as possible. The water bath surrounding the forearm was maintained at 30° C. While the 3 minute period of arterial occlusion undoubtedly caused some increase in blood flow in the skin at the temperature selected vasodilatation in the skin was far from maximal and, hence most of the rise in forearm

flow was the result of an increase in muscle flow. The exercise, which consisted of compressing a firm rubber ball every 5 seconds for 3 minutes, was sufficient to tire the muscles of the forearm and usually caused a rise in the arterial pressure in both normal and hypertensive subjects. Thirty seconds before the end of the exercise the average arterial pressure in the normal subjects was 124 mm systolic and 85 mm diastolic. In the hypertensive subjects at a similar time the average arterial pressure was 229 mm systolic and 147 mm diastolic. In the 6 normal subjects the blood flow immediately after exercise averaged 26.5 cc per minute per 100 cc of forearm, with extremes of 25 cc and 28 cc. In 3 hypertensive subjects the blood flow after exercise averaged 31.2 cc, with extremes of 30 cc and 33 cc. The average blood flow immediately following exercise was 18 per cent faster in the hypertensive than in the normal subjects. The hypertensive subjects, however, were not in as good general physical condition as the normal subjects and, though the exercise was nearly maximal for the hypertensive subjects, it did not completely tire out the muscles of the forearm in the normal persons. The experiment was, therefore, repeated in 1 normal subject in whom the blood flow after exercise had been 25 cc. This time the exercise periods were spaced at 15-minute instead of 30-minute intervals, so that after the third trial the exercise became as nearly maximal as it had been in the hypertensive group. Under these conditions the forearm flow was 33 cc, indicating that in the previous experiments on the effect of exercise in normal subjects maximal dilatation of the vessels had not been obtained. It is, therefore, concluded that under the maximal effect of the vasodilating factors present in exercise the blood flow is essentially the same in normal subjects and in patients with arterial hypertension.

Blood flow in the brain. As calculated from the O_2 differences between arterial and internal jugular blood, the blood flow through the resting brain is normal in subjects with arterial hypertension (11). These experiments do not demonstrate, however, whether the cerebral vessels can dilate to a normal degree under physiological stimuli. A progressive reduction in blood flow to the brain was selected as the best means for producing vasodilatation in the brain. It has been

demonstrated experimentally that in cats cerebral "anemia" produced by clamping the carotid arteries or by a sudden, extreme fall in blood pressure caused dilatation in the vessels of the pia (12). Wolff and Lennox showed that this vasodilatation occurred as the result of an increase in carbon dioxide content of the blood, and that it could also be produced by the introduction of other acids into the blood (13). It has also been demonstrated (14) that the cerebral vessels in man dilate when the carbon dioxide content of the blood is increased or when marked anoxemia is produced.

If cerebral anoxia is induced by the oral administration of sodium nitrite in the upright position (10), the subjects should faint at approximately the same level of arterial pressure if the vessels of the brain of the hypertensive subjects are capable under such conditions of dilating to a normal degree. If, on the other hand, they cannot dilate to the same degree as do normal vessels, fainting should occur at a higher level of arterial pressure. In the 3 hypertensive subjects tested, the patients developed signs of cerebral anoxia with arterial pressure levels of 210, 170, 140 mm systolic and 130, 100, 118 mm diastolic, respectively. In a series of normal controls, symptoms of cerebral anoxia did not develop until the systolic pressure had fallen to around 70 mm Hg. In these cases of arterial hypertension, therefore, the peripheral resistance in the brain could not be lowered to normal level by inducing cerebral anoxia.

Reduction of the arterial pressure after malaria. Two subjects with arterial hypertension were studied both before and after malaria therapy. The first case, M. F., was a 30-year-old, white female, whose arterial pressure in January, 1937, was 140 mm systolic and 100 mm diastolic. In January, 1938, the arterial blood pressure was 200 mm systolic and 130 mm diastolic. The peripheral vessels and the vessels of the fundi were normal. The heart was not enlarged. Pyelograms were negative. The clinical diagnosis was essential hypertension. The patient also had central nervous system syphilis with a paretic gold curve. She was given two courses of malarial therapy, the first in 1938, and the second in 1939, and each time the arterial pressure became much lower, both during and for some time after the

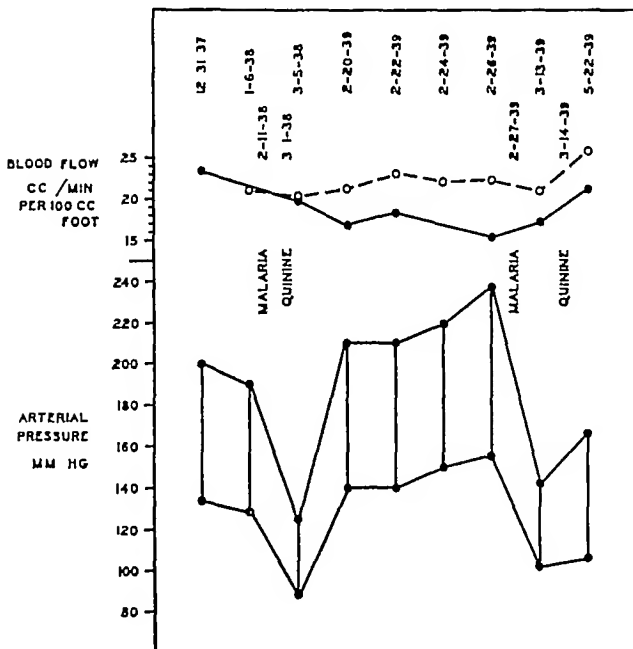


FIG. 2. THE BLOOD FLOW IN THE FOOT AT 43° C. IN PATIENT M. F.

The arterial pressure on two occasions was decreased during and for some time after a course of malaria. The blood flow in the foot did not decrease as the arterial pressure fell. Solid line = right foot broken line = left foot.

course of malaria. The blood flow in the foot showed no significant change when the arterial pressure fell (Figure 2)

In the same patient on two occasions the blood flow in the forearm after exercise was 33 cc. and 32 cc. respectively, when the arterial pressure was 240 mm. systolic and 150 mm diastolic. After the second course of malaria the blood flow in the forearm after exercise was 35 cc. when the arterial pressure was 150 mm. systolic and 110 mm. diastolic.

The second patient was a 44-year-old colored woman who had hypertension for at least 4½ years before her entry into the hospital in December, 1938. The vessels of the fundi showed marked narrowing. The peripheral vessels were diffusely thickened and the left posterior tibial pulse was not palpable. The heart was moder-

ately enlarged. The Wassermann was positive. The clinical diagnosis was essential hypertension and latent syphilis. The arterial pressure was decreased during and for some time after the course of malaria. The blood flow in the foot at 43° C decreased when the arterial pressure fell (Figure 3)

In both cases malaria caused a marked reduction in the arterial pressure at rest. In the first case the increased peripheral resistance had not resulted in any structural changes in the vessels. In the second case however permanent structural changes had occurred.

Vasomotor reactions in arterial hypertension
In subjects with arterial hypertension sensory stimuli such as noise pinch or deep breath, produced vasoconstriction in the hand and foot. These reactions were qualitatively and quantita-

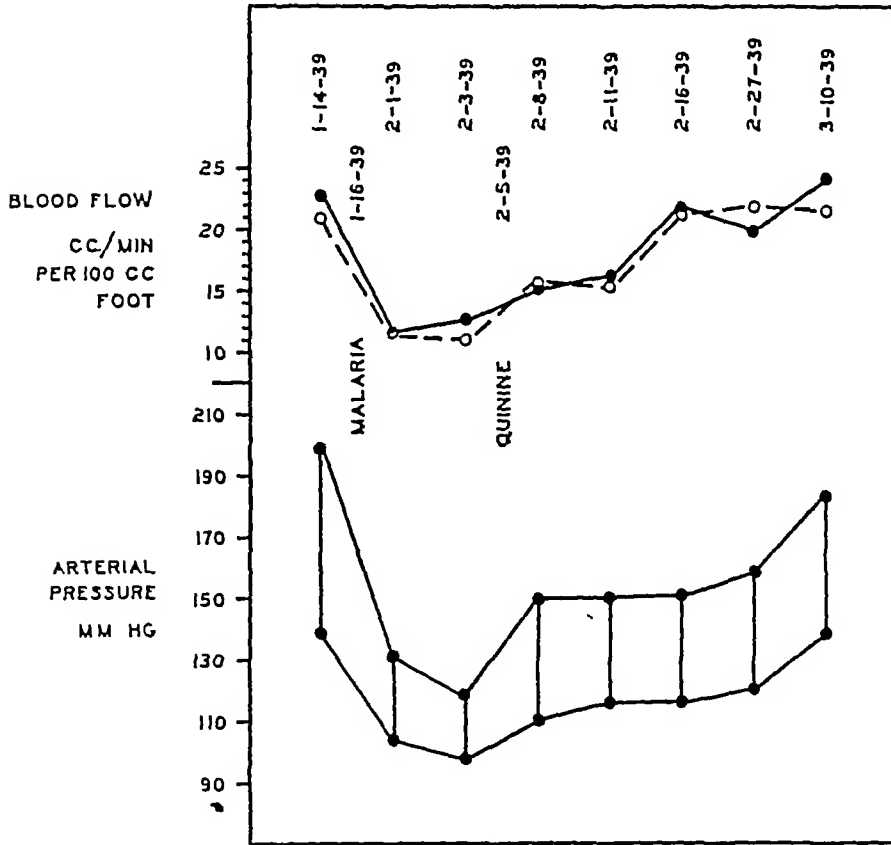


FIG 3 THE BLOOD FLOW IN THE FOOT AT 43° C. IN PATIENT D M

The arterial pressure was decreased during, and for some time after, a course of malaria. The blood flow in the foot became slower when the arterial pressure decreased. Solid line = right foot, broken line = left foot.

tively similar to those occurring in normal subjects. Vasoconstrictor responses were also obtained in 2 subjects both before and after the arterial pressure was lowered by a course of malaria. There was no difference in the vasomotor responses in the same subject at the two widely different levels of arterial pressure (Figure 4)

DISCUSSION

These experiments demonstrate that the average peripheral resistance in arterial hypertension is increased fairly uniformly in the skin, the muscles, and the brain, and that it cannot be lowered to normal levels by strong, physiological dilating agents. Studies of renal function in arterial hypertension (15) have shown that the peripheral resistance is also increased in the kidney because the blood flow in the kidney, as determined by the urea or creatinine clearance tests, is no faster

in hypertensive than in normal subjects. The measurement of the blood flow and the peripheral resistance in the splanchnic area in man is not practicable as yet. However, since in arterial hypertension the peripheral resistance is increased in the skin, muscle, brain and kidneys, and since the cardiac output is normal, one can assume that the blood flow in the splanchnic area is the same as in normal subjects, and that, therefore, the peripheral resistance in the splanchnic area is not raised to a greater degree than in the other tissues.

The finding of a uniform increase in peripheral resistance in the skin, brain and muscle is strong evidence against the neurogenic vasomotor origin of the usual clinical types of arterial hypertension. The amount of vasomotor activity varies greatly in these three tissues. Vasoconstrictor impulses play a large part in the regulation of the blood flow in the skin of the hand and foot, they play

a minor part in the regulation of the blood flow in the brain and as far as is known they play no part in regulation of blood flow in the muscle. Therefore it is very unlikely that any abnormality of the vasomotor system, or any increase in sensitivity of the vasomotor system would produce the same relative increase in the peripheral resistance in these three tissues which normally show such a great difference in vasomotor activity

formed which counteracted the effect of a vasoconstrictor substance or influence or because a vasoconstrictor substance was no longer formed, was neutralized or excreted

Although the behavior of the peripheral circulation at rest appeared to be identical in these 2 subjects the response of the vessels to vigorous dilating stimuli was different. In the case of M F, the blood flow in the foot at 43° C, and

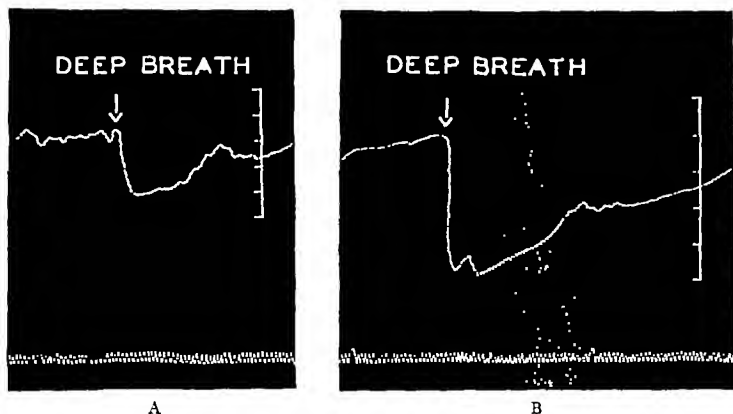


FIG. 4 VASOCONSTRICTOR RESPONSE IN LEFT FOOT OF PATIENT D M INDUCED BY A DEEP INSPIRATION

The vasomotor responses to sensory stimuli did not change when the arterial pressure fell. Note that the calibration of the recording bellows differs in A and B

A Before malaria—arterial pressure was 200 mm. systolic and 140 mm. diastolic.

B After malaria—arterial pressure was 120 mm. systolic and 98 mm. diastolic.

These experiments on the blood flow in the skin muscle and brain do not demonstrate whether the increased peripheral resistance in hypertension results from a humoral vasoconstrictor substance, or from permanent structural changes in the vessels. The subjects who were given malaria throw some light on this problem.

In the 2 patients who were given malaria, sufficient vasodilatation occurred at rest to produce a marked decrease in arterial pressure. The blood flow at rest was adequate as shown by warm extremities, full pulse and adequate output of urine. No information was obtained however as to whether this vasodilatation took place because a powerful vasodilator substance was

in the muscles of the forearm after exercise, did not decrease with the fall in arterial pressure because permanent structural changes in the vessels had not taken place. Therefore when the hypothetical vasoconstrictor substance which had caused the increased peripheral resistance and the hypertension was no longer active the vessels were able to dilate more fully in response to various physiological stimuli and to maintain the same blood flow with a lower pressure head. In the second subject D M the blood flow in the foot at 43° C decreased when the arterial pressure fell because there were permanent structural changes in the vessels. Therefore even when the hypothetical vasoconstrictor substance originally

causing the increase in peripheral resistance was not active, the vessels were not able to dilate sufficiently in response to heat to compensate for the fall in arterial pressure

In both of these cases, however, the arterial hypertension resulted from active vasoconstriction, presumably humoral in origin rather than from structural changes in the vessels because, even in the second case which had developed permanent vascular damage, sufficient vasodilatation occurred to reduce the arterial pressure when the reversible factors causing the original high arterial pressure were not active

The vasomotor responses in the hand and foot to sensory stimuli were similar both qualitatively and quantitatively in normal and in hypertensive subjects. In the 2 hypertensive subjects who showed a marked fall in arterial pressure sensory stimuli produced vigorous vasoconstriction regardless of the height of the arterial pressure. These observations are in accord with the findings of other observers (2, 3)

SUMMARY AND CONCLUSIONS

1 The increased peripheral resistance present in arterial hypertension cannot be reduced to the normal level in the skin of the hand and the foot, the muscles of the forearm, or in the brain by powerful vasodilating stimuli

2 The blood flow in the hand and foot at 43° C and in the muscles of the forearm after exercise shows no significant difference in normal and in hypertensive subjects

3 In hypertensive subjects cerebral anoxia does not reduce the peripheral resistance in the vessels of the brain to normal levels. Therefore, in postural experiments after the administration of sodium nitrite, subjects with arterial hypertension develop syncope with a much higher arterial pressure than do normal subjects

4 The finding of a uniform degree of elevation of the peripheral resistance throughout the body is strong evidence against the neurogenic origin of the usual types of clinical hypertension because the nervous vasomotor control is different in each of the tissues investigated

5 In 1 subject with arterial hypertension a marked fall in arterial pressure after malaria

produced no change in the blood flow in the foot at 43° C, indicating that no structural changes had taken place in the vessels. In a second subject the blood flow in the foot at 43° C was decreased when the arterial pressure fell indicating that permanent vascular damage had occurred. The arterial hypertension in both of these subjects originally resulted from active vasoconstriction. In 1, structural changes had later developed

6 Sensory stimuli, such as pinch, noise, and deep breath, produce vasoconstriction of similar degree in both normal and hypertensive subjects. When the arterial pressure is greatly lowered following malaria, these vasoconstrictor responses remain unchanged

7 In arterial hypertension the peripheral resistance is uniformly raised throughout the area of the greater circulation, and it is not increased in the splanchnic area to any greater extent than in other tissues

The authors wish to express their appreciation to Dr Soma Weiss for helpful guidance and criticism in this work. This investigation was carried out with the technical assistance of Miss Sophia M. Simmons, S. B.

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THE CIRCULATION IN ATHLETES¹

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The effect of exercise on the dynamics of the circulation is still not precisely known, although the subject has been extensively studied. Most investigators (1, 2, 3, 4, 5, 6, 7, 8) have given attention to cardiac size, and very little study has been directed to the functional capacity of the hearts of individuals who have engaged in athletics. The effects of acute exercise (9) on the circulation as well as the consequences of very prolonged and strenuous exercise (10, 11, 12) have been studied. The data available up to 1933 have been reviewed by Steinhaus (13). The effects on the circulation of somewhat less strenuous and prolonged exercise such as football and other college athletics, have not been properly estimated. A sufficient number of the school population in this country engage in college athletics to make pertinent an evaluation of their effects on the circulation.

In this investigation we have made certain studies of the circulation in a group of college athletes and compared them with measurements made by similar methods in a group of normal individuals engaged in ordinary sedentary occupations. These latter make up the control group. In these two groups we have made measurements of the arteriovenous oxygen differences, oxygen consumption, minute volume output of the heart, vital capacity, cardiac size, circulation time, venous pressure, arterial pressure and heart rate.

In the group of athletes there are 14 healthy males between the ages of 19 and 23 years. All were members of a college football team at the time these observations were made and most of them participated also in other athletics such as basketball, baseball and track.² The members of

this group had successfully engaged in competitive school and college athletics for periods of 2 to 9 years. They appeared to be representative of those engaged in college athletics.

The control group is comprised of 11 healthy males between the ages of 19 and 29 years who were engaged in sedentary occupations and indulged in no more than occasional mild athletic recreation. A complete history was taken and physical examination made on all members of both groups. Count of the red blood cells, estimation of the hemoglobin, Wassermann reaction in the blood, and routine examination of the urine were carried out. No abnormalities of the cardiovascular renal system were detected.

METHODS

The subjects were admitted to the hospital overnight and all observations were made the following morning while the individuals were in a basal metabolic state. They were all trained to carry out the procedure beforehand. Measurements of the cardiac output were made by the acetylene method, three samples of gas being taken, as recommended by Grollman (14) in his book on "The Cardiac Output of Man in Health and Disease," and further elaborated by Grollman, Friedman, Clark, and Harrison (15). During the measurement, the individual was sitting in a steamer chair (angle 135°) with legs extended. While resting quietly the radial pulse was counted at intervals of 5 minutes. At the end of one-half hour the acetylene-air mixture was rebreathed. Three samples of gas were then taken during each rebreathing period for estimation of the arteriovenous oxygen difference. Two periods of rebreathing were carried out on each subject. Shortly afterwards the oxygen consumption was measured with a Benedict Roth spirometer. After a short interval, the vital capacity was measured and the height and weight recorded. Then the individual rested again, now lying down. In succession sufficient time being allowed between each procedure for the patient to return to a basal metabolic state, an electrocardiogram was taken, the arm-to-tongue circulation time recorded, the venous pressure estimated, and the blood pressure measured. Finally an x-ray photograph of the heart was made at a distance of two meters.

The arm-to-tongue circulation time was estimated by the use of Decholin. 5 cc. of a 20 per cent solution were injected rapidly (1 to 2 seconds) through an 1

¹ Read by title at the Thirty First Annual Meeting of the American Society for Clinical Investigation, Atlantic City May 1 1939.

² We wish to thank Dr. William H. McCastline, University Medical Officer, Columbia University for his kindness in referring the students to us, and to express our appreciation to the members of the football team for their hearty cooperation in this investigation.

needle into an antecubital vein while the individual was lying quietly in the supine position. This was repeated in 1½ minutes after the first test had been elicited. The time was recorded from the beginning of the injection until the subject first perceived the bitter taste.

The venous pressure was measured by the direct method, using a large antecubital vein, the vein being placed on a level with the right auricle. The apparatus consisted of an L-shaped glass tube attached to a three-way stopcock, syringe and an 18-gauge needle. The apparatus was filled with a solution of sterile normal saline, a venipuncture performed, and the direct pressure readings recorded.

X-ray photographs of the heart were taken with the subjects in the standing position, in full inspiration, at a distance of two meters. Measurements of the heart were carried out by the technique of Levy (16) and estimations of volume were made as recommended by Bardien (17). In addition, measurements of the greatest transverse cardiac diameter and calculations of the cardiothoracic ratio were made.

DATA

The complete data for the athletes and normal control subjects are given in Tables I and II respectively. The measurements with the exception of cardiac size, are plotted as frequency diagrams in Figure 1.

The average value for the arteriovenous oxygen difference for the athletes was 63.9 cc, the range being from 73.9 cc to 55.2 cc. For the control group the average value was 61.5 cc, the range being from 64.8 cc to 56.1 cc (Tables I, II and III) (Figure 1).

The average value for the cardiac index (cardiac output in liters per square meter of body surface area per minute) for the athletes was 2.12 liters, the range being from 2.67 liters to 1.75 liters. For the control group, the average value

TABLE I
Measurements of certain circulatory phenomena in 14 male athletes

Name and age	Height cm	Weight kgm	Oxygen consumption liters per minute	Basal metabolic rate %	Arteriovenous oxygen difference cc	Cardiac output liters per minute	Cardiac output liters per minute	Heart rate per minute	Stroke volume cc	Cardiac area sq cm	Cardiac volume cc	Arterial pressure mm. Hg	Left ventricular work gm m. per beat	Circulation time seconds	Venous pressure cm	Vital capacity cc	Red blood count millions	Hemoglobin per cent	Stroke volume cc per kgm. wt. body wt	Left ventricular work gm m. per beat	Heart measurements		
																					Greatest transverse cardiac diameter cm	Internal thoracic diameter cm	Cardiothoracic ratio per cent
J. B. 18	171.0	89.6	214	-24	61.2	3.50	1.76	68	82	116.1	604.2	105/60	58.7	15.4	10.4	4900	5.15	108	0.60	0.68	12.6	31.0	41.6
J. B. 19	175.3	91.3	310	0	57.8	5.38	2.54	80	67	158.9	987.4	125/90	98.4	13.3	10.1	6200	5.40	128	0.73	1.08	14.4	33.0	43.6
J. C. 21	175.3	79.5	285	+1	66.4	4.31	2.20	80	54	110.8	562.3	124/80	74.9	16.0	8.4	4200	5.25	135	0.68	0.94	11.6	30.5	33.0
H. C. 21	177.0	80.1	235	-11	59.5	4.30	2.16	70	61	140.7	795.6	110/68	73.0	14.0	11.3	5100			0.76	0.91	13.9	30.1	46.2
J. C. 22	170.5	79.5	256	+5	55.9	5.12	2.67	94	54	129.5	711.3	115/50	72.0	15.4	7.9	4500	5.60	140	0.68	0.90	13.3	31.2	42.6
J. D. 21	176.3	84.1	280	-2	73.0	3.64	1.92	65	60	144.0	834.8	118/78	60.0	16.8	9.2	4600	5.50	105	0.71	0.95	14.4	31.8	45.0
G. H. 21	174.3	75.8	274	-14	73.9	3.71	1.90	66	56	118.4	622.8	110/60	72.4	12.0	8.1	5250	5.25	128	0.74	0.95	11.7	30.0	39.0
J. N. 23	177.5	73.2	235	-13	70.0	3.37	1.77	48	70	141.5	813.6	122/85	99.0	16.2	9.6	4100	5.15	127	0.96	1.35	14.3	30.0	47.7
N. P. 21	172.3	82.0	249	-10	55.5	4.50	2.28	60	75	113.2	680.4	120/85	105.0	11.6	10.1	5150	6.10	110	0.91	1.27	12.0	30.8	28.9
W. S. 23	176.5	82.0	272	0	62.0	4.71	2.33	56	84	144.4	837.4	110/70	102.8	20.1	6.1	4500	5.90	105	1.02	1.25	13.9	30.5	45.6
B. S. 23	183.5	97.5	271	-12	71.7	3.78	1.73	64	59	151.2	897.8	114/60	77.8	17.1	9.1	5300	5.70	128	0.64	0.84	14.1	32.8	42.9
A. W. 22	170.2	76.9	277	0	60.0	4.45	2.34	60	74	127.7	704.9	115/50	99.6	14.6	8.7	4300	4.50	97	0.96	1.20	13.6	30.3	44.9
H. W. 23	179.6	72.1	235	-12	55.2	4.31	2.27	55	74	132.8	732.5	108/75	106.0	15.4	9.2	4600	4.70	116	1.03	1.47	12.7	31.1	40.8
S. W. 22	182.5	74.1	274	-15	72.4	3.79	1.82	60	63	129.2	711.5	105/50	79.7	17.3	9.7	5150	5.15	125	0.75	0.94	13.2	30.6	43.1

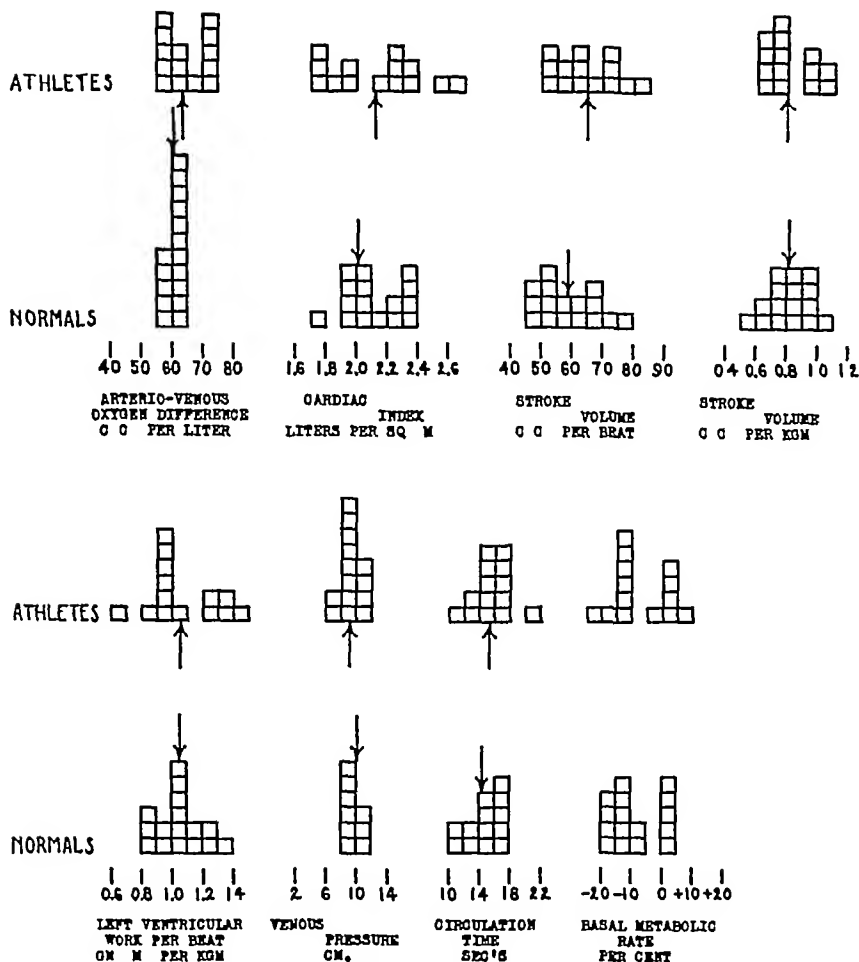


FIG. 1 MEASUREMENTS OF THE CIRCULATION IN ATHLETES AND NORMAL SUBJECTS

In this figure the data relating to the measurements of the circulation in the normal group and in the athletes are plotted as frequency diagrams (Tables I and II). Each square is a unit and represents one measurement and they are piled on top of one another when there is recurrence of that increment for the measurement in that particular group. The increments are as follows: arteriovenous oxygen difference, 5 cc. cardiac index, 0.1 liter per square meter stroke volume, 5 cc. per beat stroke volume per kilogram 0.1 cc. left ventricular work per beat, 0.1 gram. per kilogram venous pressure, 2.0 cm. of physiologic saline, circulation time, 2.0 seconds and basal metabolic rate, 5 per cent. The mean values for each group of measurements are indicated by an arrow.

TABLE II

Measurements of certain circulatory phenomena in 11 normal male individuals, by techniques similar to those used for the athletes

Name, age	Date	Height m.	Weight kg.	Oxygen consumption liters per min. per kg.	Basal metabolic rate %	Arteriovenous oxygen difference %	Cardiac output liters per min.	Cardiac output ml. per minute	Heart rate per min.	Stroke volume cc.	Stroke volume cc. per kgm.	Cardiac area sq. cm.	Cardiac volume cc.	Arterial pressure mm Hg.	Left ventricular work gm m. per beat	Left ventricular work gm m. per kgm body wt.	Circulation time seconds	Venous pressure cm.	Vital capacity cc.	Heart measurements		
																				Greatest transverse cardiac diameter cm.	Internal thoracic diameter cm.	Cardiothoracic ratio
J. R. 21	Jan. 20, 1934	176.0	83.3	261	-5	63.0	4.14	2.06	83	50	0.59	123.8	704.9	120/84	69.1	0.82			5350	12.8	30.8	41.6
	Jan. 30, 1934	176.0	83.4	283	+4	64.7	4.40	2.20	85	52	0.62	128.8	704.9	136/76	74.5	0.89			5270			
F. H. 21	Nov. 19, 1934	173.0	81.2	262	0	61.5	4.59	2.32	62	74	0.91	139.6	796.6	128/80	104.7	1.29	12.2		4800	13.8	30.1	45.8
	June 24, 1935	174.5	81.3	286	+2	63.6	4.50	2.30	66	68	0.63	134.6	753.2	130/80	97.1	1.19	15.2	11.6				
N. C. 29	Nov. 20, 1934	173.0	62.2	207	-13	63.3	3.27	1.90	68	48	0.77	133.9	791.8	120/75	63.2	1.02	14.8		5300	14.1	31.5	44.5
	Nov. 24, 1934	173.0	61.8	216	-10	64.8	3.33	1.91	65	60	0.81	138.9*	791.8	114/68	61.9	1.00	16.5		5300			
J. D. 23	Nov. 22, 1934	185.0	76.0	224	-19	63.6	3.52	1.76	60	59	0.78	149.7	675.6	118/76	77.8	1.02	16.0		5000	14.2	32.1	44.2
M. P. 19	Dec. 6, 1934	159.0	55.5	230	0	62.8	3.66	2.32	78	47	0.65	112.9	578.9	130/78	60.5	1.20	11.0	9.0	4300	11.7	28.6	40.9
W. C. 26	May 14, 1934	174.0	78.5	241	-10	64.4	3.75	1.94	80	47	0.60	144.6	639.5	130/90	70.3	0.90			4350	15.0	30.0	50.0
E. K. 24	April 8, 1933	174.5	71.5	216	-17	59.2	3.65	1.85	84	68	0.95	143.5	879.5	110/68	82.3	0.87	14.5	12.0	4400	14.6	31.2	46.8
	April 9, 1933	174.2	71.5	220	-16	59.8	3.63	2.00	53	64	0.90	144.6	837.4	104/56	69.6	0.97	16.2	9.5	4650			
	April 10, 1933	174.5	71.8	222	-15	60.4	3.65	2.00	56	68	0.92	144.7	841.1	105/58	74.5	1.04	16.8	9.8	4500			
H. B. 21	Dec. 18, 1933	176.0	65.7	232	-10	60.6	3.63	2.00	72	50	0.77	148.1	859.1	132/82	73.0	1.11	12.7	9.9	3700	14.1	29.2	48.2
R. K. 25	Nov. 7, 1935	181.3	75.4	243	-11	56.6	4.30	2.17	56	77	1.02	136.7	724.5	112/76	98.4	1.31	17.0	8.8	5150	12.8	30.6	41.8
R. W. 25	Dec. 5, 1936	178.0	74.0	247	-7	56.1	4.40	2.29	76	88	0.75	122.3	650.8	120/89	82.8	1.09	10.4	11.3	4700	13.9	30.3	45.9
C. W. 27	Jan. 16, 1937	172.2	68.9	251	0	59.6	4.21	2.33	70	60	0.87	132.3	732.0	105/68	71.0	1.03	14.1	8.7	4600	13.9	29.7	46.8

* X ray of the heart was not repeated in this case and the previous measurements were used for cardiac area and volume

was 2.09 liters, the range being from 2.33 liters to 1.76 liters (Tables I, II and III) (Figure 1)

The average value for the stroke volume in the athletes was 65 cc with the maximum and minimum values of 84 cc. and 52 cc respectively. The average for the control group was 59 cc, the range being 77 cc to 47 cc (Tables I, II and III) (Figure 1)

The average value for the stroke volume per kilogram of body weight for the athletes was 0.80 cc, the range being from 1.03 cc. to 0.60 cc. The average value for the control group was 0.81 cc, the range being from 1.02 cc to 0.59 cc. (Tables I, II and III) (Figure 1)

The average value for the left ventricular work per beat per kilogram of body weight for the athletes was 1.06 gm.m. per kgm, the range being from 1.47 gm.m. per kgm to 0.68 gm.m. per kgm. For the control group the value was 1.05 gm.m.

per kgm, the range being from 1.31 gm.m. per kgm to 0.82 gm.m. per kgm (Tables I, II and III) (Figure 1)

The average value for the venous pressure for the athletes was 9.2 cm of physiologic saline, the range being from 11.3 cm to 6.1 cm. For the control group, the average value was 10.1 cm, the range being from 12.0 cm to 8.7 cm (Tables I, II and III) (Figure 1)

The average value for the arm-to-tongue circulation time for the athletes was 15.4 seconds, the range being from 20.1 seconds to 11.6 seconds. The average value for the control group was 14.4 seconds, the range being from 17.0 seconds to 10.4 seconds (Tables I, II and III) (Figure 1)

The heart size based on measurements of the transverse cardiac diameter was considered to be within the limits of normal in every subject in both groups. The average value of this diameter

TABLE III

Average and range of values of certain measurements of the circulation in 14 male athletes and 11 normal male subjects

	Normal			Athletes		
	Average	Range		Average	Range	
		Max	Min		Max	Min
Arteriovenous oxygen difference cubic centimeters	61.5	64.8	56.1	63.9	73.9	55.2
Cardiac output, liters per square meter per minute	2.09	2.33	1.76	2.12	2.67	1.75
Stroke volume, cubic centimeters per beat	59	77	47	65	84	52
Stroke volume cubic centimeters per kilogram	0.81	1.02	0.70	0.80	1.03	0.60
Left ventricular work per beat gram meters per kilogram	1.05	1.31	0.82	1.06	1.47	0.68
Venous pressure, centimeters (physiologic saline)	10.1	12.0	8.7	9.2	11.3	6.1
Circulation time, seconds	14.4	17.0	10.4	15.4	20.1	11.6
Greatest transverse cardiac diameter, centimeters	13.7	15	11.7	13.3	14.4	11.6
Cardiothoracic ratio, per cent	45.2	50.0	40.9	42.9	47.7	38.0

for the athletes was 13.3 cm and for the control group 13.7 cm. The average value for the cardiothoracic ratio for the athletes was 42.9 per cent, and 45.2 per cent for the control group (Tables I, II and III). The greatest transverse cardiac diameter did not exceed 15.0 cm nor did the cardiothoracic ratio exceed 50.0 per cent (Table III).

While the standard lead electrocardiograms were recorded for each subject in both groups, chest leads, derived by placing the right arm electrode just within the apex and the left arm electrode in the left interscapular region were taken of 5 of the control subjects and of 13 of the 14 athletes. Normal sinus rhythm was present in all, and in each case the auriculoventricular and intraventricular conduction times were within the limits of normal. The R-T segment deviation did not exceed 1 mm and the T-waves in leads I and II were upright and more than 3 mm in amplitude. The amplitude of the QRS complexes exceeded 5 mm in two or more leads in every instance. Only 1 individual of each group showed a significant axis deviation (J N, Table I and N C, Table II) which was to the left. The chest lead was considered normal in every subject in which it was obtained.

DISCUSSION

It appears that there is no significant difference between the average values of the athletes and the control normal group for the arteriovenous

oxygen difference, cardiac index (cardiac output in liters per square meter of body surface area per minute), stroke volume per kilogram of body weight, and the left ventricular work per beat per kilogram of body weight. It is also apparent that the individual variations for these measurements are within the accepted limits of normal in every case for both groups. Our values for these measurements of the circulation are in agreement with those obtained by Grollman (14) on a group of 50 young, normal adults. The average value for the stroke volume of the athletes, however, is found to be appreciably greater than that of the normal subjects. This difference is apparently due to a difference in the average size (body surface area and weight) of the individuals as the cardiac index and the stroke volume per kilogram of body weight, as stated above, show no significant differences in the two groups.

The average values for the circulation time and venous pressure in the two groups show no significant difference. Tarr and his associates (18) found that the normal value for the circulation time (Decholin) in their group fell between 100 seconds and 160 seconds. In our normal subjects, the individual estimations ranged from 10.4 seconds to 17.0 seconds. With one exception (W S, Table I, 20.1 seconds), the athletes fell in the normal range. The measurements of the venous pressure, cardiac output and cardiac size of this individual were, however, within the limits of normal.

The work of the left ventricle per beat has been plotted against the cardiac volume (Figure 2). In a statistical study Starr and his associates have shown that in the presence of normal circulatory function the work of the left ventricle is proportional to the size of the heart (19, 20). They have defined a zone of normal circulatory function (zone CD-EF) from their data. In those individuals falling below the line CD the work per beat is not commensurate with heart size and patients suffering from congestive heart failure have been shown to lie in this area (Starr (19, 20, 21), Stewart (22, 23, 24)). In this study we found that every individual in both the group of athletes and the normal (control) group fell within the zone of normal circulatory function (Figure 2).

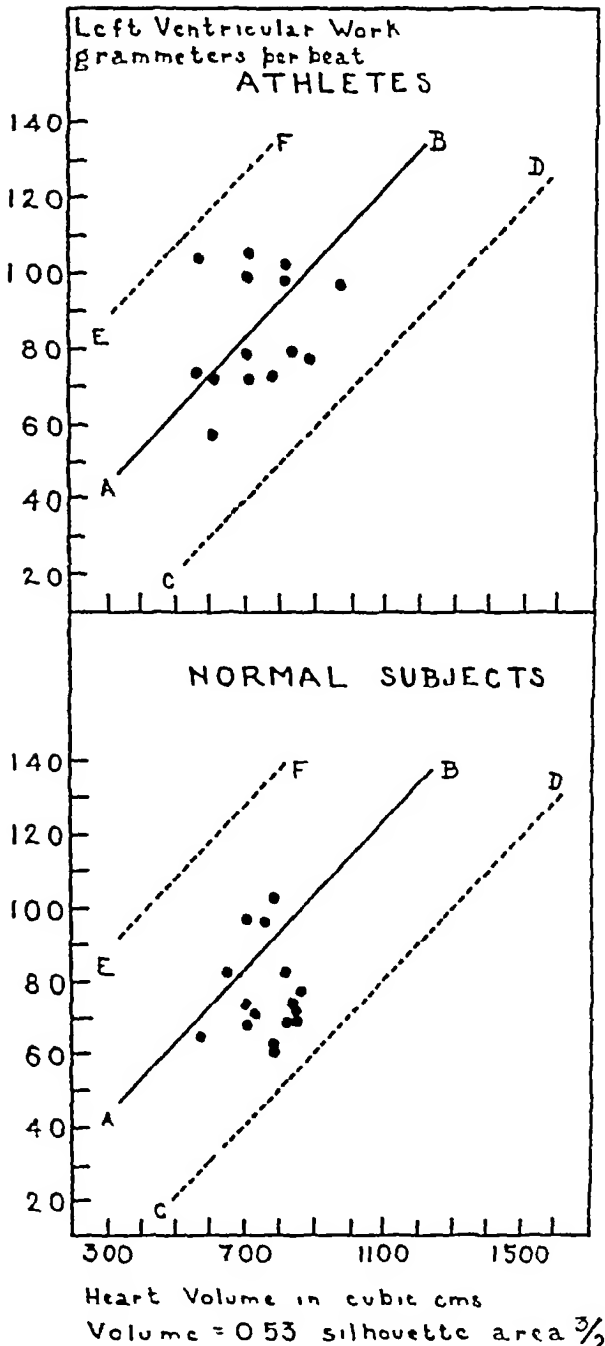


FIG. 2. LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME

The data from Tables I and II relating to the work of the left ventricle per beat in both the normal group and the athletes are plotted against the corresponding cardiac volumes. Line AB represents the best line, the regression of the work on the area, defined by Starr, Collins and Wood (19) on the basis of a statistical treatment of data from a control group of cases. Lines CD and EF are placed by these authors at a distance of

The ratio of transverse cardiac diameter to the chest diameter has been used in this study as a measurement of heart size in addition to the heart volume-work correlation. While our findings of a heart of normal size in the group of athletes is in agreement with the work of other observers (1, 3, 4, 8, 25), it is not to be inferred from our data that cardiac enlargement does not occur in individuals engaged in more strenuous and prolonged types of exercise such as marathon running.

In this group of athletes, all the electrocardiograms were considered normal. Bramwell and Ellis (12) report "variations" in the form of the P-wave, T-wave, and R-T segment, as well as prolongation of the P-R interval in electrocardiograms of Olympic athletes. The auriculoventricular conduction time was within the limits of normal in all of our cases and no distinct differences in the form of the records between the control group and athletes were observed. The differences between our findings and those observed by Bramwell and Ellis may be explained on the basis of the types of athletes which were studied in the two reports.

Finally, it should be pointed out again that all of our observations were made while the individuals were in a basal metabolic state. Although these studies reveal no significant differences in the functional capacity of the hearts of the athletes, as compared with the normal subjects who were leading a sedentary life, it does not necessarily follow that the individuals used as controls can perform the same amount of work as efficiently as the athletes.

SUMMARY

1. Measurements of the arteriovenous oxygen difference, oxygen consumption, minute volume output, stroke volume, vital capacity, cardiac size,

twice the standard deviation from AB. It appears from their observation that an individual falling within the zone CD-EF has a normal circulatory function. That is to say, the work of the heart is commensurate with its size, on the other hand, they found that the values relating to patients who had suffered from cardiac decompensation fell in a zone below CD. Each closed circle in the above figure represents a measurement in that group as indicated. It is apparent that all individuals of both the normal group and the athletes fall well within the zone of normal circulatory function (zone CD-EF).

circulation time and venous pressure, arterial pressure and heart rate, were made of 14 college athletes and also of 11 healthy, young adult males who were living a sedentary life and who served as controls. These objective measurements were carried out under basal conditions.

2 With one exception, there was no significant difference between those made on college athletes and those derived from the control group. The stroke volume of the athletes was slightly larger. This difference appears to be related to body size, since the stroke volume per kilogram of body weight and the cardiac index for the two groups are approximately the same.

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SERUM LIPOIDS AND PROTEINS IN HYPERTHYROIDISM¹

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It has been stated frequently that the level of serum cholesterol is as valuable an index of hyperthyroid activity as the basal metabolic rate. An examination of the literature, however, reveals controversial material to support this dictum. The present study was undertaken to determine how closely the level of serum cholesterol is related to the degree of thyroid activity and whether it is a useful criterion in the diagnosis of hyperthyroidism. It was thought of importance also to know if changes in the level of serum cholesterol after the administration of iodine might help in diagnosis in those questionable hyperthyroid patients whose basal metabolic rates do not respond to the ingestion of Lugol's solution. In addition to the cholesterol basal metabolic rates serum fatty acids, phosphatides and proteins of 65 patients with symptoms of hyperthyroidism have been determined at frequent intervals to learn (1) whether there is an inverse relationship between basal metabolic rate and serum cholesterol, lipid phosphorus fatty acids and proteins, (2) if these serum constituents increase when the basal metabolic rate falls after the administration of iodine and (3) if thyroidectomy always increases these components. The data on only 43 patients are presented in the tables because the other 22 patients were not completely studied. There were about 430 samples of blood sera in which the three lipid fractions and proteins were determined during the 3 or more years that many of the patients have been followed.

Hurxthal, Hunt and co-workers have studied the largest group of patients (23, 24, 25, 26, 38). Although they have employed a cholesterol colorimetric method described by Bloor in 1922 their results are reliable because they have devoted considerable attention to normal values. They have placed the low limit of normal for their method at 120 mgm per cent, and the high limit at 300

mgm per cent (26). In the study of patients with hyperthyroid disease these authors have found cholesterol values in a large majority of patients which were close to their low limit of normal. The rest of the values were distributed about equally on both sides of this figure (23). These authors have considered the possible effect of malnutrition on the serum cholesterol and have decided that it is of minor importance (23). They conclude that serum cholesterol is low in hyperthyroidism and that there is a relation between the severity of the disease and the level of cholesterol (23). Recent studies by Boyd indicate that total lipoids and phospholipids, as well as cholesterol, tend to be low in hyperthyroidism (7, 8, 9, 10, 11). Thus these recent studies on a large number of patients support the view that severe degrees of hyperthyroidism are usually associated with low levels of serum cholesterol (4, 16, 41, 45, 47).

It is, however, probable that there are a considerable number of patients with severe hyperthyroidism who do not have low serum cholesterol or other fractions of the lipoids. A number of workers have reported that in patients with hyperthyroidism the blood or serum cholesterol may be within normal limits (1, 3, 15, 28, 30, 44, 52), or sometimes high (17, 22, 29, 39, 40, 51). That there is no clear-cut relation between basal metabolic rate and cholesterol is probably due to many factors. Inaccuracies and differences in methods failure to choose relatively clear-cut examples of hyperthyroidism or to study enough patients, account for some discordant results. Furthermore, in evaluating data, such factors as state of nutrition and hemoconcentration and the condition of the nervous system of the patients have been neglected.

Most of the previous workers have measured only one form of blood lipid usually total cholesterol. The problem of cholesterol methods is an old and controversial topic. This matter has been

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discussed by the authors (36) in a paper on the measurement of cholesterol in which it was pointed out that the colorimetric methods were unreliable and subject to errors varying from minus 18 to plus 76 per cent. Schoenheimer (48), and also Kirk, Page, Van Slyke and co-workers (27, 43) have recently reinvestigated cholesterol methods and have adopted a method dependent on digitonin precipitation. Even in Bloor's laboratory where the colorimetric and nephelometric methods were revised again and again, a digitonin precipitation method has been developed (42, 53). In justice to some colorimetric data it should be recognized that certain workers with a flair for colorimetry, who have carefully devised and checked their own standards and ruled out complicating precipitates and colors, have probably achieved approximately accurate results. In many of the papers on hyperthyroidism not enough details as to technique and range of normal values for the method have been presented to permit evaluation of the results.

MATERIAL AND METHODS

The 43 patients included in this study, except as otherwise indicated, exhibited most of the symptoms of hyperthyroidism and had basal metabolic rates of more than plus 30 per cent, as estimated from the lowest value obtained from repeated tests. As will be described in detail under DATA, the clinical features of exophthalmos, malnutrition, tremor, restlessness, degree of cardiac enlargement and vasomotor phenomena including sweating, flushing, pulse irregularities and rate, have been carefully evaluated. Evidences of disorders of the endocrine glands other than the thyroid were sought.

The mental and emotional status, degree of vasomotor instability, and neurological status of each patient were studied in the search for underlying nervous system disorders that could not readily be considered as secondary to overactivity of the thyroid (13). Previous investigators have pointed out that the nervous and mental symptoms produced by excessive amounts of thyroid secretion consist of a fine muscular tremor, awareness of rapid heart beat, perspiration, restlessness, irritability, and a feeling of fatigue. In the present study the above symptoms and a moderate degree of anxiety and fear of the illness have been considered as being due to hyperactivity of the thyroid. Other mental symptoms such as marked depression in mood, states of agitation often over trivial or imaginary troubles, severe anxiety and delusions or persecution have been considered as evidence of an underlying nervous system disorder not secondary to overactivity of the thyroid.

Additional evidence in favor of or against the extra

thyroid origin of the nervous system disorders was sought in the past history and in the course of the symptoms after thyroidectomy. The story of mental disorders, such as encephalitis and manic depressive psychosis, long before the onset of the characteristic symptoms of hyperthyroidism, and the persistence of these symptoms long after the basal metabolic rate had fallen to zero or below, were considered as supporting the extra thyroid origin of such symptoms. Stress has been laid on these vegetative and central nervous system disorders because recent studies of the authors indicate that elevated serum lipoids are frequently found in patients with these conditions (20).

All of the metabolic studies were made in the morning when the patients were in the post-absorptive state. The basal metabolic rates were measured with the Benedict-Roth apparatus under the standard conditions which have been described by Benedict, Dubois and others. The venous blood samples were usually taken after this test or on the following day. They were collected under oil, centrifuged with anaerobic precautions, and the serum analyzed according to methods previously described for cholesterol (5, 36), fatty acids (32, 34), lipid phosphorus (31, 36), proteins, albumin and globulin (14).

Histopathological studies of the glands were made independently in the Department of Pathology of the Yale University School of Medicine.²

DATA

In Tables I, II, and III are charted the symptoms of 43 patients as well as the basal metabolic rate and serum cholesterol determined in most instances before administration of Lugol's, after Lugol's but just before thyroidectomy, 1 to 8 weeks after thyroidectomy, and 4 to 40 months after thyroidectomy. Table I includes 13 uncomplicated hyperthyroid patients, Table II, 9 patients who had a good recovery, and Table III, 21 patients who only had a partial recovery after thyroidectomy. In Tables II and III, the open circles (O) mark the 21 patients with marked disorders of the vegetative and central nervous system, including patients with diabetes and marked vasomotor disturbances resembling those described in a previous paper (37). The remaining 9 patients denoted by crosses (X) suffered from a variety of disorders in addition to thyroid disease. Three (A55758, 27339, A57648) had peculiarities of physical makeup suggestive of complex glandular disorders. Five (96354, 625, A43137, 70165, 84321) had long histories of repeated recurrence and removal of thyroid tissue. One (A39091) had cardiac decompensation, hypertension and minimal symptoms of hyperthyroidism.

Enlargement of the thyroid has been graded as + when the gland was slightly palpable, ++ when large, +++ large with bruit, and ++++ when the enlargement was extreme. Cardiac enlargement has been

² We are indebted to Dr Harry M Zimmerman of the Department of Pathology for re-evaluation of this material in connection with the present paper.

TABLE I
Uncomplicated hyperthyroid patients

Case number age, and sex	Symptoms of hyperthyroidism							Complications							Before Lugol's		After Lugol's before operation		1-4 weeks post operation		4 or more months post operation	
	Enlarged thyroid	Cardiac		Tremor	Motor restlessness	Exophthalmos	Skin	Brain stem disorders							Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol
		Enlargement	Rate					Disproportionate vasomotor instability	Prothyroid disorders	Disorders continue after 24 hr. bed rest + 10	Parkinsonian tremor	Miscellaneous	Glaucoma	Neuropathies								
per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	
23516 ● F 18	++	0	150	++	++	+	++	0	0	0	0	0	0	—	+61	78	+7	145	+9	200	-8	181
A56897 ● M 23	++	0	120	++	+	+	+	0	0	0	0	0	0	—	+85	125			+2	200	-12	175
A44525 ● F 21	+	+	110	++	+++	0	++	0	0	0	0	0	0	—	+72	113	+7	151	-4	240		
A65117 ● F 19	++	+	130	++	++	++	++	0	0	0	0	0	0	N	+105	114	+49	141	+9	196		
A82477 ● M 22	++	0	125	++	+	+	+	0	0	0	0	0	0	—	+84	190	+11	220	+8	259	-7	220
A55387 ● F 19	++	0	130	++	++	0	++	0	0	0	0	0	0	+	+35	117	+13	164	-8	202	-28	176
A88333 ● M 35	+++	0	120	++	+	++	++	0	0	0	0	0	0	—	+69	116	+35	129	+2	220		
B850 ● F 31	++	0	123	++	++	+	++	0	0	0	0	0	0	—	+59	115	+15	142	+2	178		
A59169 ● F 37	+	+	125	++	+	0	++	Exophthalmos developed 1 month post-operative							+49	91	+23	148	-18	154	-23	149
6525 ● F 43	+++	+	110	+++	++	+	++	0	0	0	0	0	0	—	+60	124	+11	212	-7	272		
A57234 ● M 45	+	0	100	++	+	+	+	0	0	0	0	0	0	N	+55	134	+41	190	+16	248		
A65283 ● M 21	+	+	88	+	+	0	+	0	T	0	0	0	0	—	+51	130	+20	148	+2	157	-13	134
A50122 ● F 20	+	0	108	++	+	+	+	0	0	0	0	0	0	—	+69	151	+21	170	+7	277		

* Lugol's continued

evaluated either by physical examination or x ray and graded + to +++ Pulse has been recorded as the rapid rate when the patient was resting in bed soon after hospitalization. If an individual had auricular fibrillation or had been digitalized, the pulse rates have been omitted. Under tremor + indicates a perceptible fine tremor of the extended tongue or fingers of the hand, ++ an easily recognizable tremor and +++ severe tremor obviously involving the whole body. This fine tremor of hyperthyroidism has been contrasted with the coarse Parkinson like tremors which have been listed under brain stem disorders. Motor restlessness has been difficult to grade, but slight overactivity is described as +. When activity was sufficient to make it difficult to keep a patient in bed it is graded +++ and when the

patient required a special room for management ++++. Any perceptible exophthalmos or lid lag has been listed as +. Where all the signs of exophthalmos were present but not marked, ++ have been used and +++ for degrees beyond this.

Under "skin" have been included flushing, sweating and satiny texture. Different degrees of flushing and sweating have been described as + to ++++. In certain patients the flushing and the vasomotor disturbance were disproportionately great in comparison with weight loss, cardiac enlargement, thyroid involvement and height of basal metabolic rate. Such outstanding vasomotor instability has been evaluated under brain stem disorders and has been called "disproportionate vasomotor instability." Conditions which have returned or persisted

TABLE II
Hyperthyroid patients with good recovery after thyroidectomy

Case number, age, and sex	Symptoms of hyperthyroidism							Complications							Before Lugol's		After Lugol's before operation		1-8 weeks post operation		4 or more months post operation					
	Enlarged thyroid	Cardiac		Tremor	Motor restlessness	Exophthalmos	Skin	Brain stem disorders				Disproportionate vasomotor instability	Prethyroid disorders	Disorders continue after B.M.R. below +10	Parkinsonian tremor	Miscellaneous	Glandular	Nutrition	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol
		Enlargement	Rate					Toxic psychosis	Nutrition																	
per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.	per cent	mgm. per 100 cc.																			
A535100 F 40	+	0	120	++	++++	0	++	++	D	0	0	Toxic psychosis	-	+33	102	+22	232	-2	306	-6	263					
A561710 F 66	++	++	Ir	+++	++	0	++	+	D	My D	+	0	0	--		+56	189	+10	228	-17*	435					
A495610 F 43	++	0	100	+	+	+	++	++	0	VI G	0	G	?	-	+41	172	+27	218	+12	260	-6	183				
A55753X M 42	+	+	Dec A F	+	0	0	+	0	C	C	0	0	?	Ob.	+56	157	+32	245	+47	233	+11	197				
A751570 F 57	+	+	A.F	+	+++	0	++	++	0	Aa. My	0	G Aa.	0	--	+64	124	+27	171	+18		-3	238				
A433300 F 60	+	0	100	++	+	0	++	++	V.I	V.I	0	0	Ov	--	+36	160	+14	176	-27*	603						
A676900 M 52	++	0	118	+	+	0	++	++	0	0	0	0	0	-	+42	165	+15	107	-9	105	-9	260				
A555100 F 69	++	0	160	+	0	0	+++	+	Aa.	V.I	0	G Aa.	0	--	+72	150	+69	181	+13		+8	248				
96351X F 43	++++	++	130	+++	+	0	++	0	T	Hp C	0	Aa. Hp.	-		+38	106	+39	123	+12	212						

* Desiccated thyroid prescribed for thyroid deficiency symptoms

Definition of symbols

- Acr Acromegaly
 A.F Auricular fibrillation
 An Anemia with a red count below 3,800,000
 As Arteriosclerosis as indicated by hardened or tortuous radial arteries or tortuous or nicked retinal blood vessels
 C Cardiac condition such as enlargement or decompensation necessitating the use of digitalis
 D Mental and physical depression
 Dec Cardiac decompensation
 E I Emotional instability
 Hp Hypertension of 160/100 or above
 G Severe glycosuria for which insulin was used
 Hys Hysterectomy
 Ir Irregularities in pulse other than auricular fibrillation
 M D The overactivity of manic depressive psychosis
 M General weakness, myasthenia.
 Ob Overweight of an extreme degree in which the distribution of fat is eccentric
 Ov Bilateral ovariectomy
 T Previous thyroidectomy or exacerbations and remissions of hyperthyroidism.
 V I Vasomotor instability of an extreme variety

TABLE III

Hyperthyroid patients with partial recovery after thyroidectomy

Case number, age, and sex	Symptoms of hyperthyroidism							Complications							Before Lugol's		After Lugol's before operation		1-6 weeks post operation		4 or more months post operation		
	Enlarged thyroid	Cardiac		Tremor	Motor restlessness	Exophthalmos	S.B.H.	Brain stem disorders					Miscellaneous	Glandular	Nutrition	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol	Basal metabolic rate	Serum cholesterol
		Enlargement	Rate					Electrocardiogram abnormality	Fibrinolytic disorders	Disorder continues after B.M.R. below +10	Parkinsonian tremor												
A44132 O F 47	+++	0	120	++	+++	0	++	0	0	D My	0	0	0	---	per cent +78	mgm. per 100 cc. 160	per cent +87	mgm. per 100 cc. 141	per cent -11	mgm. per 100 cc. 211	per cent -13	mgm. per 100 cc. 204	
A43251 O F 54	++	+	110	++	+++	++	++	0	M.D.	M.D.	0	Osteoporosis	0	0	---	per cent +57	mgm. per 100 cc. 200	per cent +22	mgm. per 100 cc. 272	per cent +13	mgm. per 100 cc. 235	per cent -5	mgm. per 100 cc. 223
A22133 O F 50	+	0	115	+	0	0	+	0	M.D. My	M.D. My	0	Am.	0	---	per cent +67	mgm. per 100 cc. 105	per cent +22	mgm. per 100 cc. 191	per cent +11	mgm. per 100 cc. 222	per cent -23	mgm. per 100 cc. 153	
A58463 O F 48	++	+	110	++	++	0	++	++	M.D.	My	0	Hp.	---	per cent +47	mgm. per 100 cc. 145	per cent +10	mgm. per 100 cc. 195	per cent +8	mgm. per 100 cc. 243		mgm. per 100 cc. 219		
P 162 O F 60	++	+	95	++	++++	0	++	+	Am. M.D.	Am. M.D.	+	Psychosis with Am.	---	per cent +16	mgm. per 100 cc. 169	per cent -8	mgm. per 100 cc. 274		mgm. per 100 cc. 208		mgm. per 100 cc. 208		
27343 X F 40	+	+	90	+	+	+	++	+	E.L.	E.L.	0	Ob.	+	per cent +18	mgm. per 100 cc. 134	per cent -4	mgm. per 100 cc. 245	per cent -2	mgm. per 100 cc. 202		mgm. per 100 cc. 202		
625 X F 39	++	+	90	++	++	0	+	+	E.L. T	E.L.	0	?	+	per cent +22	mgm. per 100 cc. 125	per cent +5	mgm. per 100 cc. 153	per cent +16	mgm. per 100 cc. 160	per cent -1	mgm. per 100 cc. 156		
A44734 O M 33	+++	0	120	++	++	+	++	0	M.D.	M.D.	0	0	0	---	per cent +40	mgm. per 100 cc. 176	per cent +4	mgm. per 100 cc. 243	per cent -7	mgm. per 100 cc. 191		mgm. per 100 cc. 191	
A58279 O F 42	++	+	100	++	+	+	++	++	E.L.	E.L.	0		---	per cent +31	mgm. per 100 cc. 154	per cent +18	mgm. per 100 cc. 196	per cent +4	mgm. per 100 cc. 232	per cent -11	mgm. per 100 cc. 241		
A 6235 O F 61	++	+	110	+++	++	++	+++	+++	E.L.	O.E. L	+	G	Acc.	---	per cent +58	mgm. per 100 cc. 194	per cent +87	mgm. per 100 cc. 490	per cent +80	mgm. per 100 cc. 490	per cent +46	mgm. per 100 cc. 600	
A43137 X F 37	+++	++	125	++	++	+	++	0	T	My E.L.	0		---	per cent +46	mgm. per 100 cc. 172	per cent +21	mgm. per 100 cc. 195	per cent -16	mgm. per 100 cc. 205*		mgm. per 100 cc. 205*		
A39001 X F 41	0	+++	Dec.	+	++	0	++	+++	O.E. L	O.E. L	0	Hp. Am.	N	---	per cent +89	mgm. per 100 cc. 203	per cent +4	mgm. per 100 cc. 244	per cent +2	mgm. per 100 cc. 350*		mgm. per 100 cc. 350*	
A44764 O F 25	+++	+	130	++	+	+++	+++	0	E.L. T	E.L.	+		---	per cent +73	mgm. per 100 cc. 125	per cent +28	mgm. per 100 cc. 177		mgm. per 100 cc. 216	per cent +2	mgm. per 100 cc. 216		
A48979 O F 29	++	0	102	++	+	+	++	+++	D. E.L.	D. E.L.	0	Hyp.	N	---	per cent +34	mgm. per 100 cc. 171	per cent +17	mgm. per 100 cc. 206	per cent -1	mgm. per 100 cc. 230	per cent -2	mgm. per 100 cc. 190	
70143 X F 58	+	+	108	++	+	0	+	0	E.L. T	E.L.	0		---	per cent +48	mgm. per 100 cc. 140	per cent +36	mgm. per 100 cc. 153	per cent +34†	mgm. per 100 cc. 177		mgm. per 100 cc. 177		
84321 X M 58	++	++	A.F.	++	+	++	+	0	T	C.	0		---	per cent +34	mgm. per 100 cc. 102	per cent +16	mgm. per 100 cc. 118	per cent +18	mgm. per 100 cc. 143	per cent +31	mgm. per 100 cc. 110		
82482 O F 63	++	+	100	++	+	0	++	0	E.L.	E.L.	++	Am.	---	per cent +36	mgm. per 100 cc. 178	per cent +28	mgm. per 100 cc. 219	per cent -2	mgm. per 100 cc. 265		mgm. per 100 cc. 225		
A54118 O F 55	+	++	95	+	+++	+	++	++	E.L. G	E.L. G	0	Am. Hp.	---	per cent +58	mgm. per 100 cc. 190	per cent +18	mgm. per 100 cc. 240	per cent +10	mgm. per 100 cc. 220	per cent -5	mgm. per 100 cc. 202		
A67185 O F 31	+	0	110	+	+	++	+++	++	M.D.	M.D.	0		---	per cent +32	mgm. per 100 cc. 141	per cent +20	mgm. per 100 cc. 134	per cent +2	mgm. per 100 cc. 213	per cent -10	mgm. per 100 cc. 202		
A37548 X F 43	+++	+	100	++	++	++	++		D My	My		Ob.		per cent +73	mgm. per 100 cc. 109	per cent +29	mgm. per 100 cc. 140	per cent +1	mgm. per 100 cc. 255		mgm. per 100 cc. 255		
A11909 O F 45	++	+	120	++	0	0	+++	++	D My	My	0	My	---	per cent +37	mgm. per 100 cc. 197	per cent +21	mgm. per 100 cc. 197	per cent -11	mgm. per 100 cc. 270		mgm. per 100 cc. 270		

* Desiccated thyroid prescribed for thyroid deficiency symptoms

† Lugol's continued.

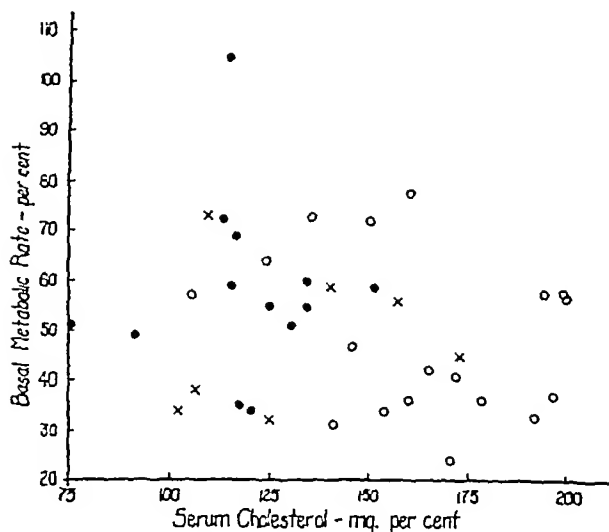


FIG 1 BASAL METABOLIC RATES AND SERUM CHOLESTEROLS BEFORE TREATMENT

Solid circles represent patients free from complicating disorders (Table I) Open circles represent patients with marked disorders of vegetative and central nervous

system. Crosses designate patients with a variety of disorders such as peculiar physical makeup or repeated exacerbations of hyperthyroidism.

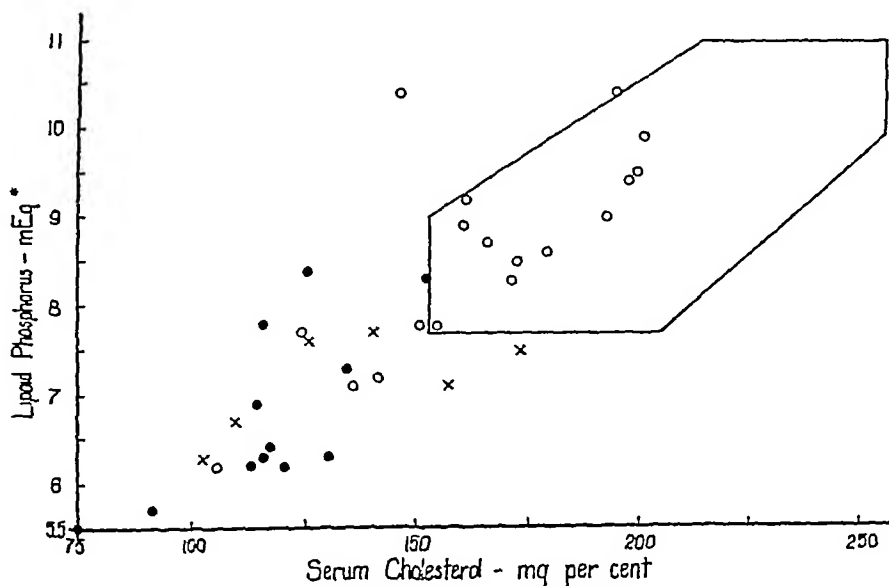


FIG 2. SERUM CHOLESTEROL AND LIPOID PHOSPHORUS BEFORE TREATMENT

The same symbols have been used as in Figure 1 The hexagon indicates distribution of similar data on 95 normal men and women

* Instead of mEq unit should be mgm. per cent.

more than a month after thyroidectomy have been marked in the column 'Disorders continue after B.M.R. below plus 10 per cent.'

Nutrition has been rated as N when normal or average —when approximately 10 per cent of weight had been lost, and ——— when emaciation was obvious, with wrinkled skin, hollow cheeks and general weakness. For people who remained distinctly well nourished, + has been used.

The serum cholesterol and basal metabolic rate are compared in Figure 1 the cholesterol and lipid phosphorus before treatment are shown in Figure 2 Figures 3 and 5 show the changes in cholesterol and basal metabolic rate, in fatty acids and in basal metabolic rate before and after Lugol's. In Figure 4 the increases after thyroidectomy of cholesterol and lipid phosphorus above the values before iodine therapy are illustrated.

The pathological studies of the thyroid glands of the

patients in Tables I, II and III have been summarized in Table IV

RESULTS

In Figure 1 it is apparent that cholesterol and basal metabolic rate were not inversely proportional. In the 13 patients with uncomplicated hyperthyroid disease represented in the chart by black circles, all cholesterol were under 151 mgm. per cent, but below this limit there is little evidence

of extreme vasomotor instability or, in addition, disorders of the nervous system tended to have normal lipoids

Figure 3 and the tables reveal that all but 6 of the 37 patients studied before and after Lugol's administration had decreases of 7 to 65 per cent in basal metabolic rate and increases in serum cholesterol of 10 to 100 mgm. per cent. The cholesterol of 3 (A44243, A67482, A61909) of

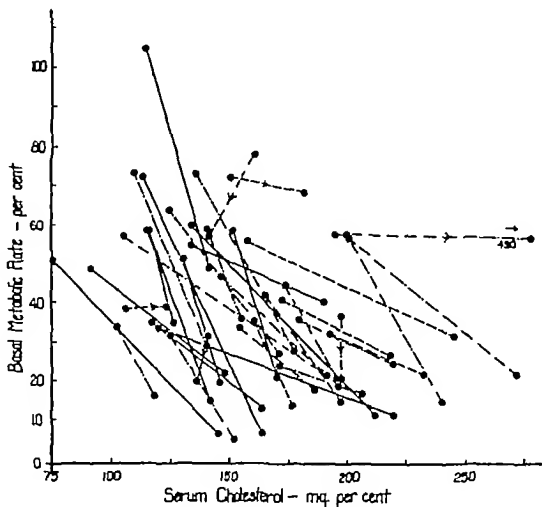


FIG. 3 EFFECTS OF IODINE THERAPY ON CHOLESTEROL AND BASAL METABOLIC RATE OF 37 PATIENTS

Lines connect the values for cholesterol and basal metabolic rate before Lugol's administration and after Lugol's administration just before operation. Solid lines for uncomplicated patients of Table I broken lines for patients who made a good recovery after thyroidectomy (Table II) lines with a dot and a dash for patients who had a partial recovery after thyroidectomy (Table III). The 6 patients represented by lines which do not follow the slope of the majority of the lines have been marked by arrows and are discussed in the text.

of an inverse relationship with basal metabolic rate. This lack of relationship can be attributed somewhat to the great variability in the levels of serum cholesterol

Inspection of Figure 2 reveals that all of the patients who were free from complications had serum cholesterol and phospholipoid values well below normal. In contrast, patients with evi

dences of extreme vasomotor instability or, in addition, disorders of the nervous system tended to have normal lipoids

the 6 patients represented by lines marked by arrows which do not follow the direction of the majority of the lines, did not increase cholesterol of 3 others (A58510 96354 A9255) increased but the basal metabolic rates did not change more than 3 per cent. The 3 patients whose cholesterol did not increase all had initial cholesterol ranging between 141 and 197 mgm.

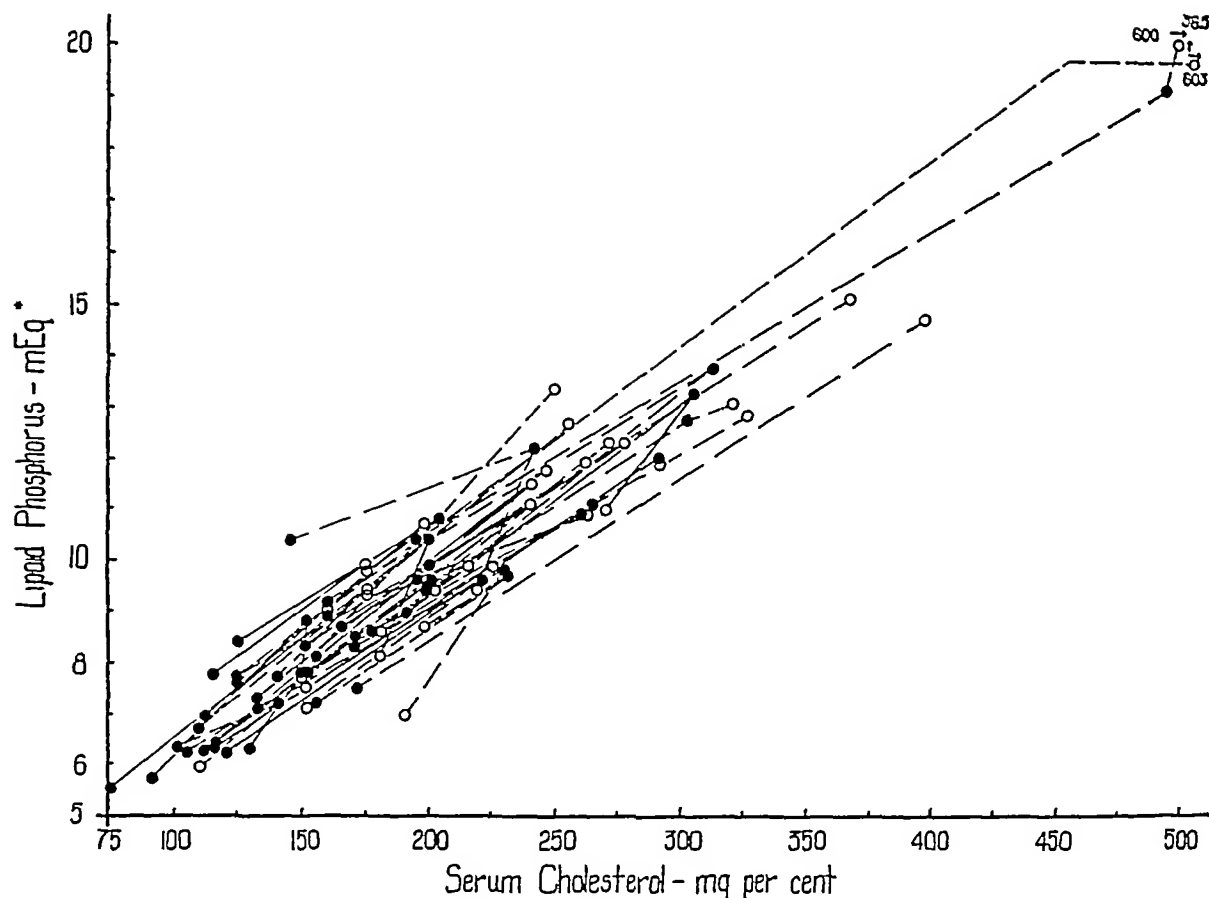


FIG 4 EFFECTS OF THYROIDECTOMY ON SERUM CHOLESTEROL AND LIPOID PHOSPHORUS OF 32 PATIENTS

Lines similar to those in Figure 3 connect the values before Lugol's administration and 1 to 8 weeks after thyroidectomy, and in 18 patients 4 or more months after thyroidectomy. The final study on each patient, whether 1 to 8 weeks or 4 or more months after thyroidectomy, is represented by an open circle.

* Instead of mEq unit should be mgm. per cent.

per cent, and exhibited symptoms of vasomotor instability, myasthenia, depression and anxiety of a marked degree. These symptoms have been shown by the authors (20) to be correlated with a lability in serum lipoids which may account for the apparent lack of response of serum cholesterol to the administration of Lugol's solution. They were also patients who were only slightly improved by thyroidectomy. Pathologically, A44243 had a colloid goiter with only a little hyperplastic tissue, A67482 also had little hyperplasia which was irregularly distributed and there were islands of epithelial metaplasia, the gland of A61909 was indistinguishable from the glands of uncomplicated patients in Table I. Of the 3 patients whose basal metabolic rates were not appreciably lowered by Lugol's solution, 1 (A9255) had acromegaly. The gland of the acromegalic (A9255) had only

scattered areas of hyperplastic tissue, no high columnar epithelium and, in most areas, fairly normal looking colloid. There was a considerable amount of interstitial hyaline. The second (96354) had the most grossly enlarged thyroid of all the patients in the tables and histologically the gland contained a good deal of colloid but also had local areas of hyperplastic tissue. The third (A58510), 69 years old, had arteriosclerosis, vasomotor instability, and glycosuria for which she required insulin before thyroidectomy. An adenoma was found without much evidence of hyperplastic tissue.

Subtotal thyroidectomy was followed in all patients by a rise in serum cholesterol and a fall in basal metabolic rate. The cholesterols before the administration of Lugol's and 1 to 8 weeks after operation have been considered quantitatively

in only 30 of the 43 patients. In 4 patients (A75157, A58510, A54754, A54764) the follow-up study was 4 or more months after operation, and in 5 patients (A56471, P1692, 27339, A54754, A39091) there was no study before the administration of Lugol's. Eight weeks after thyroidec-

tomies. The thyroids of these 4 patients were not remarkable with the exception of 625 whose gland contained many bizarre cells that could not be classified, and there was only a small amount of hyperplasia. Since the normal variation in cholesterol has been found to be 59 mgm per cent

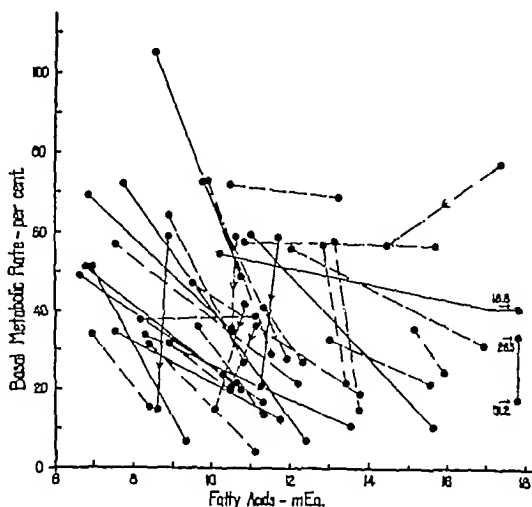


FIG. 5 EFFECTS OF IODINE THERAPY ON THE BASAL METABOLIC RATE AND SERUM FATTY ACIDS OF 37 PATIENTS

The lines representing the patients in Table I, II and III are similar to those in Figure 3. The same distance represents 3 milliequivalents of fatty acids as was used for 50 mgm. per cent of cholesterol in Figure 3. These amounts are considered to be equivalent because the normal range of cholesterol varies from 150 to 250 mgm. per cent and the normal range for fatty acids from 9 to 16 milliequivalents (19, 33, 34) and because in a normal subject cholesterol may change by as much as about 50 mgm. per cent and fatty acids by about 3 milliequivalents (35). Arrows mark the lines which do not follow the general trend and show a decrease in fatty acids rather than an increase after Lugol's.

tomy, 2 patients (A45359, A43137) who showed marked symptoms of thyroid deficiency, 2 patients (A67824, 70165) who were still taking Lugol's solution, and the acromegalic patient (A9255) all showed sufficiently atypical symptoms to be excluded from the quantitative comparison of cholesterol before Lugol's and 8 weeks after operation. In the 30 typical patients serum cholesterol increased by 59 to 169 mgm. per cent in 26 subjects and increased by 27 to 40 mgm per cent in 4 in

(35), 26 of the 30 patients exhibited significant increases in cholesterol. The average of the 30 cholesterols before Lugol's was 139 and 1 to 8 weeks after operation 235 mgm per cent.

This comparison of the serum cholesterols before Lugol's and 1 to 8 weeks after thyroidectomy was made at this interval because 11 of the 26 patients studied more than 4 months after thyroidectomy have shown a tendency for the cholesterol to rise abruptly soon after operation and then to

fall and assume a level after more time elapsed. The first patient in Table I, the first 4 patients in Table II, and the first 6 patients in Table III exhibited this leveling off after the noticeable post-operative rise. In 1 patient the table does not give space for the blood studies continued some years which made the leveling process more obvious. A56471 (Table II), after her cholesterol had risen to 435 mgm per cent, was given one grain of desiccated thyroid per day for about a year and a half. Thyroid was stopped completely and 8 months later the cholesterol was 237 mgm per cent. A patient (A78256) not listed in this paper had a serum cholesterol of 383 mgm per

TABLE IV *

Comparison of histological findings in the thyroids of patients in Tables I, II and III

Case number	Character of epithelium		Amount of colloid	Hyperplasia	Remarks
	High columnar	Papillary projections			

UNCOMPLICATED HYPERTHYROID PATIENTS, (I)

28216	+++	+++	-	+++	
A56897	++	+	+	++	
A44838	++	++	+	+++	
A65412	+++	+++	-	+++	
A82427	++	+++	-	+++	
A65387	+++	+++	-	+++	
A58933	++	++	+	++	
8850	+	+++	+	++	
A59140	++	+++	+	++	
6525	+++	++	-	+++	
A67824	+++	++	+	+++	
A65385	+++	+++	-	+++	
A56133	++	+	+	++	

HYPERTHYROID PATIENTS WITH COMPLICATIONS WHO MADE GOOD RECOVERY AFTER THYROIDECTOMY, (II)

A53510	++	++	+	++	Adenoma
A56471	++	+	++	++	Adenoma
A49564	+	++	++	++	
A55758	+	-	++	+	Adenoma
A75157	++	++	+	++	
A45359	+++	+++	+	+++	Carcinoma †
A67690	+++	-	++	+	
A58510	++	+	++	+	Adenoma
96354	++	+	+++	++	

* Customary pathological terms have been used in describing the thyroid glands. Comparative degrees of pathological change have been graded + to ++++. Dashes indicate almost entire absence of characteristic at head of column.

† Adenoma in which there was sufficient distortion of structure to suggest carcinoma.

TABLE IV—Continued

HYPERTHYROID PATIENTS WITH COMPLICATIONS WHO MADE PARTIAL RECOVERY AFTER THYROIDECTOMY, (III)

Case number	Character of epithelium		Amount of colloid	Hyperplasia	Remarks
	High columnar	Papillary projections			
A44243	-	+	+++	+	Colloid goiter
A45261	-	++	+	+	
A22433	-	+	+++	+	Epithelial metaplasia
A58658	++	++	+++	++	Adenoma
P1692		+	+	+	Fetal adenoma
27339			+	+	Fetal adenoma
625	-	+	++	+	Bizarre cells
A54754	++	++	++	++	
A59279	+++	+	++	++	
A9255	-	+	+++	+	Hyalinized tissue
A43137	++	++	-	++	
A39091	-	+	++	+	Epithelial metaplasia
A54764	+	+	+	+	
A48979	-	+++	++	+	
70165	++	++	+	++	
84321	++	+++	-	+++	Epithelial metaplasia
52492			+	+	Adenoma
A54415	+	++	+	+	
A67482	++	+	++	+	Epithelial metaplasia
A57648	++	+	++	+	
A61909	++	+	+	++	

cent and symptoms of hypothyroidism soon after thyroidectomy. After a short course of desiccated thyroid, the symptoms of hypothyroidism disappeared and did not return and the serum cholesterol was maintained at about 250 mgm per cent. The correlation between serum cholesterol and thyroid deficiency has been discussed in another paper (21). However, the evidence given in relation to the level of cholesterol before and after Lugol's solution, before and after thyroidectomy, and before and after the administration of desiccated thyroid, demonstrates a decided correlation between the level of serum cholesterol and the activity of the circulating thyroid hormone in the individual.

Serum lipid phosphorus values were so closely related to serum cholesterol values that one may be predicted from the other. This relationship is obvious in Figure 2 in which the initial values for each component are shown. The relationship is also clear in Figure 4 in which the points representing each component in 32 individuals before

Lugol's, 1 to 8 weeks after thyroidectomy and in 18 individuals 4 or more months after operation, have been connected by lines. All these lines have approximately the same slope. The increases in serum cholesterol during the interval of Lugol's administration and 1 to 8 weeks after operation were accompanied by proportionate increases in lipid phosphorus. In those patients studied 4 or more months after thyroidectomy, there was equally good correlation. If the cholesterol decreased and assumed a lower level, the phosphatides behaved similarly. The same relationship existed between lipid phosphorus and cholesterol before and after Lugol's administration. The lipid phosphorus of 29 of the 34 patients studied at this interval increased, the phospholipids of only 1 (A45359) diminished. In 4 individuals the changes were less than 0.3 mgm per cent of lipid phosphorus or within the experimental error of the method.

Values of serum fatty acids were proportional to those of serum cholesterol but not as precisely as were those for lipid phosphorus. The fatty acids were studied before and after Lugol's in 37 patients and are shown graphically in Figure 5. The fatty acids of 31 patients rose after Lugol's while, as has been shown, the cholesterol of 34 of the 37 patients rose after Lugol's. Analysis of the exact figures for fatty acids, which are not included in the tables for the sake of brevity, revealed that 11 of the 37 patients had increases exceeding 3 milliequivalents of fatty acids and 11 of the 37 had increases of cholesterol exceeding 50 mgm per cent. This analysis and a comparison of Figures 3 and 5 in which the scale for fatty acids and cholesterol were chosen to be equivalent, show that the increases in fatty acids were of approximately the same order of magnitude as the increases in cholesterol.

The fatty acids increased after thyroidectomy in all patients except A54754. He was inadequately studied with no determination of fatty acids before Lugol's or 1 to 8 weeks after operation. All of the 30 patients whose cholesterol was compared before Lugol's administration and 1 to 8 weeks after thyroidectomy had increases in fatty acids during this interval. Twenty three had increases in fatty acids greater than 3 milliequivalents the variation in a normal subject (35). The greatest rise in these 30 patients was 8.7 milli-

equivalents of fatty acid. Twelve of 26 patients studied 4 or more months after thyroidectomy exhibited the tendency for the fatty acids to fall and level in the same manner as cholesterol leveled.

There was no consistency in the changes of total proteins, albumin and globulin as a result of Lugol's and for this reason no data or figures are given. Of 37 determinations of total protein before and after Lugol's 4 patients had no change, 16 patients had an increase, and 17 a decrease in total proteins. Of 21 determinations of albumin 2 patients had no change, 9 had an increase and 10 a decrease in albumin. In the 21 examinations of globulin, there was no change in 4, an increase in 6, and a decrease in 11.

The serum proteins of 28 of 32 patients whose proteins were studied before Lugol's administration and after operation increased by 0.2 to 2.2 per cent. The average for the total proteins of 32 patients before Lugol's was 6.7 and after thyroidectomy 7.3 per cent. The serum albumin of 22 of the 24 patients studied before Lugol's and after operation increased by 0.1 to 1.8 per cent and decreased in 3 patients. Of 24 comparisons of serum globulin determinations before Lugol's and after thyroidectomy 1 patient had no change, 17 had increases of 0.1 to 0.6 per cent and 6 had decreases in globulin. The highest serum protein albumin or globulin after operation has been used in this comparison because no tendency for the proteins to level some time after operation was obvious. However, there were excluded from these comparisons all post-operative studies which were made when a patient had symptoms of thyroid deficiency or was being given iodine for a prolonged time. The changes in total and fraction proteins were so unimportant and insignificant that the level of serum proteins has no value in the diagnosis of hyperthyroidism.

A comparison of the histological findings in the thyroids of the patients in Tables I, II and III has been presented in Table IV. It can be seen that the glands from patients with uncomplicated hyperthyroidism in Table I were uniformly hyperplastic while those from patients with complications Tables II and III, were with 4 exceptions variable in appearance. It proved relatively simple to grade the amount of hyperplasia in the first group, but proved difficult in the other 2 groups due to the variability of structure in dif-

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ferent parts of the glands. For example, from a single gland in one section marked evidences of hyperplasia were present, while in the next there was adenomatous tissue and in a third islands of epithelial metaplasia. Some of these differences have been indicated in the table under remarks.

The glands from the patients with complications who made a good recovery did not differ consistently from those who made a partial recovery. It is noteworthy, however, that 5 of 9 who made a good recovery had adenomata, while only 4 out of 21 were found in those who made a poor recovery.

Although the grading of hyperplasia proved difficult, much less was found in the latter 2 groups than in the first. In fact only 1 patient from Table II and 1 from Table III were rated +++ as against 8 out of the 13 in Table I. Little colloid was found in the glands of patients from Table I, more was found in those from Tables II and III. Thus the pathological studies failed to support differences between the 3 groups of patients in only 4 cases, A49564, A54754, 84321, and A61909.

DISCUSSION

Examination of the three tables and of Figure 2 reveals that measurement of the initial serum lipoids was of uncertain value in determining whether or not the patients would respond well to Lugol's or thyroidectomy. In Figure 2 the cholesterol and phospholipoid figures of the uncomplicated patients were low but the mean for the whole group would obviously be close to the lower limit of normal. This wide distribution of values suggests one explanation of the discordant reports in the literature, for any studies involving less than 15 or 20 patients might well have been weighted in one direction or the other. In this study, however, the majority of patients, 30 out of 43, presented features which previous studies in this laboratory have demonstrated to be commonly associated with high serum lipoids. Patients with extreme forms of vasomotor instability, as for example certain diabetics described by Man and Peters (37), frequently have high lipoids. Another group of patients with complex vegetative system and emotional disorders indicative of hypothalamic and basal ganglia dysfunction also tends to have high lipoids (20). A55758,

an obese pyknic male, had an initial cholesterol of 157 mgm per cent. It has been shown that the cholesterol of a pyknic male usually lies in the upper limits of the normal range, 200 to 250 mgm per cent (19). This man may, therefore, have had a real reduction in cholesterol, although this component was not below the average normal range. All of the patients above the low limits of normal in Figure 3 belonged in one or more of these categories. When these factors are considered, it is reasonable to assume that the thyroid secretion has either reduced the lipoids from levels considerably above the normal limits or that its effects are counteracted by factors which tend to elevate the lipoids.

At present the factors which control the level of blood lipoids are not sufficiently well understood to warrant the use of lipoid values in determining the advisability of thyroidectomy. In the patients with clear-cut symptoms of hyperthyroidism and no complicating features, all of whom had low lipoids, the problems of diagnosis and prognosis were relatively simple and were not further clarified by knowledge of the level of the lipoids. On the other hand, in the complicated patients, the level of the initial cholesterol was no criterion of the patient's response to thyroidectomy. For example, A53510 who had a cholesterol of 192 mgm per cent before iodine therapy, an atypical clinical picture, and a poor response to treatment with iodine, improved after thyroidectomy as rapidly and completely as did the patients with uncomplicated hyperthyroidism. A44243, with a cholesterol of 160 mgm per cent, presented a similar picture, yet after thyroidectomy experienced very slow improvement and at the end of 2 years was not well enough to work. In contrast, A22433 with atypical symptoms that made the diagnosis uncertain, had a cholesterol of 105 mgm per cent, a high basal metabolic rate, and a good response to iodine. Yet this patient experienced only a moderate temporary relief from symptoms, with later recurrence, and after more than 2 years has shown little improvement. Her condition can scarcely be attributed to recurrence of or persistence of hyperthyroidism because her basal metabolic rate remains at about minus 23 per cent. That the initial level of serum cholesterol before Lugol's administration is of little assistance in the diagnosis of hyperthyroid-

ism has been pointed out recently by Boyd and Connell who have studied plasma lipoids in patients with anxiety syndromes (11)

The data presented in the tables have been carefully analyzed to see whether the reaction of the individuals serum cholesterol to Lugol's administration may be of aid in deciding whether to operate. The cholesterol of 13 of the 37 patients studied before and after iodine therapy did not increase by as much as 30 mgm per cent. Of these 13 2 (A45359, A43137) for several years since thyroidectomy have required desiccated thyroid for the relief of myxedema. Four (A65385, 96354 70165, 84321) had had earlier exacerbations of hyperthyroid symptoms and had been given Lugol's for a time some months or years previously. In 3 patients the basal metabolism decreased definitely after iodine although the cholesterol did not rise significantly. One (A58933) was a severely malnourished leptosomic male. His cholesterol normally was probably low (19) and the small increase after Lugol's from 116 to 126 mgm per cent may be associated with his expected low normal level. The cholesterol of 2 females (8850, A56133) who clinically exhibited adequate responses to thyroidectomy increased only from 115 to 142 and from 151 to 170 mgm. per cent, but the cholesterolemia after Lugol's may have been close to the normal for them. Four (A44243 625 A67482 A61909), of whom all but 625 did not have a satisfactory response in basal metabolic rate to iodine therapy had very little improvement after thyroidectomy and for 6 months and longer remained fatigued and nervous although the pronounced exophthalmos of the right eye of A67482 improved decidedly.

Just as the basal metabolic rate does not always fall when Lugol's is given the cholesterol may not always rise because the hyperthyroid condition may be progressing to such an extent that iodine is unable to do more than neutralize the effect of the increasing hyperthyroidism. A patient A82380, not in this series gives an example of a fall in serum cholesterol parallel with a progression of hyperthyroid symptoms when she was not taking iodine. She had a basal metabolic rate of plus 1 and a cholesterol of 148 mgm per cent but disproportionate hyperthyroid symptoms. When given Lugol's for 2 months she had little

symptomatic relief and the basal rose to plus 10 per cent. No lipoids were determined at this date. Five weeks after Lugol's was stopped her basal was plus 25 per cent and the cholesterol had fallen to 129 mgm per cent. She was given Lugol's and the basal fell to plus 8 per cent while the cholesterol rose to 175 mgm. per cent. In the intermediate period without iodine therapy and with progression of hyperthyroid symptoms, the cholesterol fell from 148 to 129 mgm per cent. In comparing cholesterol before and after Lugol's, one may well take into account an aggravation in the hyperthyroid condition which would tend to lower the serum cholesterol and thus cancel any effect of iodine therapy on the level of serum lipoids.

The diagnostic value of serum cholesterol determination is slight but it does supply corroborative evidence of the more important clinical observations and behavior of the basal metabolic rate. It may be of use when accurate basal determinations cannot be obtained. If the patient does not improve clinically, if the basal does not fall, and if the cholesterol fails to rise after Lugol's thyroidectomy probably will not be effective. Whether the reverse can be stated with equal assurance is still uncertain. Three patients (A2875, P1237, P1529), not in this series with slightly increased basals who did not conform to the hyperthyroid picture were all given Lugol's. Their cholesterol increased from 139 to 200, from 194 to 266 and from 185 to 269 mgm per cent. For clinical reasons they did not have thyroidectomies and therefore as test cases they were quite unsatisfactory. Two patients (A53920 A61169) not included in this series with extreme vasomotor instability diabetes requiring insulin tachycardia, arteriosclerosis and who lacked the typical symptoms and signs of hyperthyroidism had basal metabolic rates of plus 29 and plus 24 per cent. The cholesterol of both fell equivocally after Lugol's from 312 to 279 and from 270 to 265 mgm per cent respectively. The basal responses were quite as uncertain. Again operation seemed unjustifiable and therefore the significance of the relation between atypical lipid response to iodine and atypical symptoms must remain a subject for speculation. That increases in serum cholesterol are the usual responses of hyperthyroid patients to iodine therapy has been pointed out by Hurx-

thal (24), Bartels (1, 3), and by Nicholls and Perlzweig (41) who also found increases in fatty acids. What the behavior of the serum cholesterol of a non-hyperthyroid patient is after iodine administration does not seem to have been completely investigated. Turner (50) found no significant change in the serum cholesterol of 9 out of 10 non-hyperthyroid patients given 2 grams of potassium iodide daily for 4 to 6 weeks. The cholesterol of the exceptional patient, a female diabetic, rose from the abnormally high average of 384 mgm per cent before potassium iodide to an average of 434 mgm per cent during the iodide administration.

No correlation was found between the height to which the lipoids rose after thyroidectomy and the degree of recovery. Two of the patients whose initial lipoids were low showed after thyroidectomy little rise in serum cholesterol and other lipoids, although the basal metabolic rates fell below zero, and they experienced marked clinical improvement. One of these patients (A59140) is also remarkable because of the development of a unilateral exophthalmos in the course of convalescence. The other patient (A65385) illustrates how slight the rise in cholesterol may be even when the basal metabolic rate falls to minus 13 and there is definite clinical improvement. The lipoids of 11 of 26 patients studied more than 6 months tended to level off after the usual post-operative rise. This fall in lipoids occurred in the absence of a concomitant rise in basal metabolic rate. The first patient in Table I, the first 4 in Table II, and the first 6 in Table III belong in this group. This fall in cholesterol several months after thyroidectomy has been previously reported by Hurxthal (24, 25).

The 21 patients who presented symptoms of nervous system disorder as well as hyperthyroidism were partly relieved of symptoms by thyroidectomy but their subsequent courses progressed or failed to progress independently of the level of basal metabolic rate or serum lipoids. Patients A44243, A45261, A22433, and A54754 illustrate various responses of this type.

The 5 patients with sufficiently severe diabetes to require insulin for control before thyroidectomy varied considerably after operation. A75157 and A58510, who did well after thyroidectomy, no longer required insulin, A49564 and A54415 were

improved but continued to require insulin. These patients presented a complex variety of symptoms but they all had in common vasomotor instability and other nervous system disorders which were possibly due to a primary disorder of the vegetative centers in the brain stem. Patient A9255 represents an extreme form of the breakdown of these regulative mechanisms. The pituitary gland may have played a rôle but the persistent and gross tremor and extreme vasomotor instability indicate the presence of a disorder in the brain stem.

It is noteworthy that the subsequent comparative investigation of the pathology of the thyroid glands of these patients lends support to their classification into at least 2 groups. The glands from the clinically uncomplicated patients, Table I, proved to be uniformly and moderately ++ to severely +++ hyperplastic, while those from patients with complications presented numerous variations in structure and usually less clear-cut evidences of hyperplasia (Table IV).

Difficulties were encountered in evaluating the degree of malnutrition from the history and the general appearance of the patient. The nutritional condition of the patients in the present study is of interest because there is considerable evidence that malnutrition induced by a variety of conditions may lower the serum lipoids (33). On the basis of the story of weight loss and clinical appearance, all but 9 of the 43 patients had lost weight markedly. When the per cent of weight loss was plotted against cholesterol in mgm per cent, no relation between these two values could be discovered. On the other hand, the evidence derived from the protein and albumin studies indicates that in some patients these substances suffered a depletion similar, if less severe in degree, to that found in malnourished patients. Bartels (2, 3) has reported a depletion below normal levels in total proteins, and in some hyperthyroid patients, in albumin before treatment. In his 59 cases 3 months after thyroidectomy the total proteins and albumin were normal. He attributes these findings to the state of the liver rather than to the nutritional status or to changes in hemoglobin concentration. Brown and Mecray (12) have also reported a rise in proteins after thyroidectomy. In 32 of the patients in Tables I, II, and III, the mean value for the total proteins

before operation was 6.7 per cent and after convalescence rose to 7.3 per cent. Patients who required desiccated thyroid or prolonged iodine therapy during convalescence were excluded from this comparison. The latter figure approximates that found in well nourished people by Bruckman, D'Esopo and Peters (14, 46). This rise in proteins may have been due to the simultaneous improvement in nutrition, but it may also be affected in part by hemoconcentration. The results of the measurements of the albumin and globulin fractions are not sufficiently clear-cut to differentiate between these two possibilities. Gibson has reported that "in 25 cases of hyperthyroidism the blood volume was increased above normal on an average of 5.45 per cent" (18). In 14 subjects the increase occurred in both protein fractions and consequently it is possible that hemoconcentration may have been responsible for the change. The fact that the albumin increased more markedly than the globulin indicates that hemoconcentration may not have been important and that the small rise was probably due to improvement in nutrition.

A correlation between the 3 lipid fractions—total fatty acids, phosphatides and cholesterol—with thyroid activity in a single individual is apparent from the analysis of the results. Changes in cholesterol were accompanied by proportionate alterations in phospholipoids and by slightly more variable changes in total fatty acids. If the proteins are used as a criterion of blood volume, the increases in lipoids greatly exceed any diminution in blood volume. The post thyroidectomy protein was 109 per cent of the initial protein value while the post thyroidectomy cholesterol was 169 per cent of the initial value. It must be stressed that from one patient to another the height of the basal metabolic rate was not proportional to the level of serum lipoids but the close correlation between the different lipoids was obvious in each subject as an individual. Nicholls and Perlzweig (41) found similar changes in cholesterol and fatty acids of hyperthyroid patients. Boyd and Connell (8, 9) have pointed out that the neutral fat was diminished less than the total lipid, total cholesterol, free cholesterol or phospholipoids. However, Boyd in an earlier article (6) in which he described his lipid techniques stated that "of all the lipids, neutral fat and cholesterol ester were

found to be the most variable." That neutral fat might vary greatly can be understood readily because in his method total fatty acids and total cholesterol are determined together. From this value must be subtracted the determined total cholesterol, the computed cholesterol ester, fatty acids, the phospholipoid fatty acids computed as two thirds of the phospholipoid in order to obtain the figure for neutral fat. This fraction is therefore subject to the summation of errors. The observations of Soskin and Mirsky (49) that in a patient who refused thyroidectomy the daily feeding of 230 grams of fat supplemented by 3 grams of cholesterol for 23 days resulted in remission of hyperthyroid symptoms, are of interest in relation to the problem of lipemia in hyperthyroidism. The results of earlier workers, the data given previously and the observations in a companion article (21) on hypothyroidism show that the level of serum lipoids is closely related to overactivity and underactivity of the thyroid gland.

CONCLUSIONS

The basal metabolic rate, serum cholesterol, lipid phosphorus, titrated fatty acids, total proteins, albumin, globulin and pathology of thyroid glands of 43 patients with symptoms of hyperthyroidism have been studied. Blood studies were made at frequent intervals which, as often as possible, were before Lugol's administration, after iodine but before thyroidectomy, 1 to 8 weeks and 4 or more months after operation.

The level of serum lipoids before iodine therapy was not related to the height of the basal metabolism and was of little value in predicting the degree of improvement after thyroidectomy. In 13 uncomplicated patients the cholesterol before Lugol's was below 151 mgm per cent, the lower limit of the normal range. Complex factors other than the thyroid, such as extreme vasomotor instability and vegetative nervous system disorders, have been considered in relation to the initial level of the cholesterol.

After Lugol's administration the cholesterol in 34 of 37 patients increased 10 to 100 mgm per cent and the basal metabolic rate of 34 of these 37 patients fell 7 to 65 per cent. This rise in cholesterol has been evaluated as a criterion to be used in considering the advisability of thyroidectomy.

After thyroidectomy the cholesterol of all the 43 patients increased. In 26 of 30 patients studied before Lugol's and 1 to 8 weeks after operation, the increases were more than 59 mgm per cent, and therefore more than the limits of variation in a normal individual. The cholesterol of 11 patients rose sharply soon after thyroidectomy and then fell to a constant level 4 or more months after operation. The height of the rise was not related to the degree of improvement and recovery.

Changes in cholesterol were accompanied by proportionate alterations in phosphatides and by somewhat less precise changes in fatty acids.

No consistent changes in total proteins, albumin or globulin occurred during the administration of iodine. In 28 of 32 patients the serum proteins after thyroidectomy were higher than before Lugol's by 0.2 to 2.2 per cent. The serum albumin exhibited a greater tendency to increase than serum globulin. The relation of these increases to the state of nutrition of the patients has been discussed.

The patients whose hyperthyroidism was clinically uncomplicated had glands that were uniformly hyperplastic and contained relatively small amounts of colloid. The patients whose clinical conditions were complicated had thyroids with variable histopathological pictures. There was in general less hyperplastic tissue. Many glands had adenomata, while others contained islands of epithelial metaplasia, bizarre cells, and relatively large amounts of colloid.

It would have been impossible to collect this material if it had not been for the clinical services of Dr. Paul Lavietes, Dr. C. L. Robbins, Dr. Alexander Winkler, and Dr. Kalmen A. Klinghoffer under whose care were many of the patients.

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THE DECREASE OF GASTRIC SECRETION WITH ADVANCING YEARS FURTHER OBSERVATIONS

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In previous papers we (1) and others (2) have shown that gastric secretion decreases with advancing years. This finding, however, has been based on averages of many examinations in different people and no accurate information is available as to what happens in the individual. Does a gradual decline of secretion take place in everyone or do some people undergo an abrupt deflection, while others preserve their gastric juice unchanged into old age? In the attempt to answer these questions we previously reported the results of standard histamine tests repeated in the same individuals after an interval of five years (3). The findings were of interest but, unfortunately the five-year period appears to have been too brief to yield conclusive results. Recently it has been possible to re-examine five essentially normal people whose gastric secretions were explored over ten years ago. The data from these tests which seem much more significant than those of the previous series, are herewith presented.

PROCEDURE

The procedure was as far as possible a duplication of the original examination. The subjects were in the ward overnight and were under basal conditions. The standard histamine test used by us for many years was employed. The dose of histamine was the same on successive tests and all examinations were made by the writer. The total secretions were collected over successive ten minute periods by continuous aspiration, and in the charts volume refers to the amount of such ten minute yields. Acidity refers to the total acidity of successive specimens measured in the usual way.

RESULTS

Case 1 (Number 167640) Pi male, age 40 had been operated on for gall stones. His gastro-intestinal tract showed no lesion. At the time of our first test he felt well and has been well ever since. The first examination was made on November 22, 1927 and the second on

March 5 1939 after an interval of over eleven years (age 52). The results are shown in Figure 1. The successive curves both for acid and volume are practically identical.

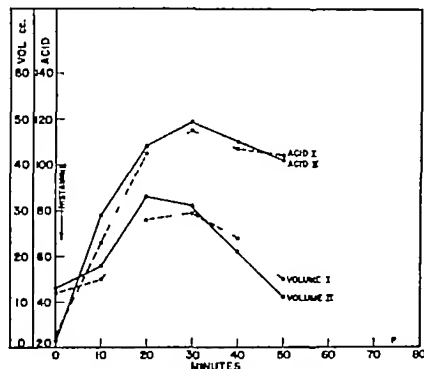


FIG. 1 OBSERVATIONS IN CASE 1

Case 2 (Number 175600) Ha, a 27 year-old male, was originally examined as a normal control. The first test was made on May 14 1928 the second on February 23 1939 after an interval of practically eleven years (age 38). He was perfectly well. The results (Figure 2) show practically identical curves on the first and second examinations. Of special interest is the sudden rise in the volume curve on both occasions. Cases 1 and 2 illustrate how constant the gastric secretory response may be if successive tests are done under similar conditions.

Case 3 (Number 175711) Sc, a 32-year-old male, had no medical disease. He was examined on May 18 1928 and again on February 10 1939 after an interval of eleven years (age 44). On the first test he showed somewhat unusual findings, namely relatively low acidity with very large volumes of gastric secretion, and it was interesting that we obtained practically the same results eleven years later. There is a slight difference in the shape of the volume curves but the maximum secretion during a ten minute period is practically the same (32 cc., 30 cc.).

Case 4 (Number 114604) Ka a 42 year-old male with vague psychoneurotic symptoms was examined on November 11 1927 January 17 1935 and December 2, 1937

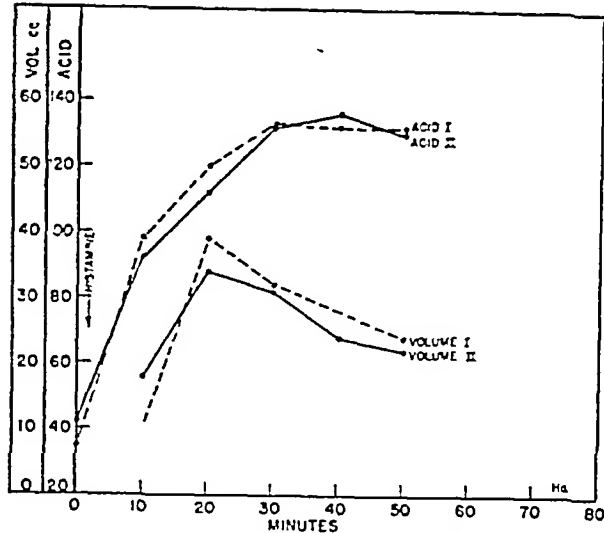


FIG 2 OBSERVATIONS IN CASE 2

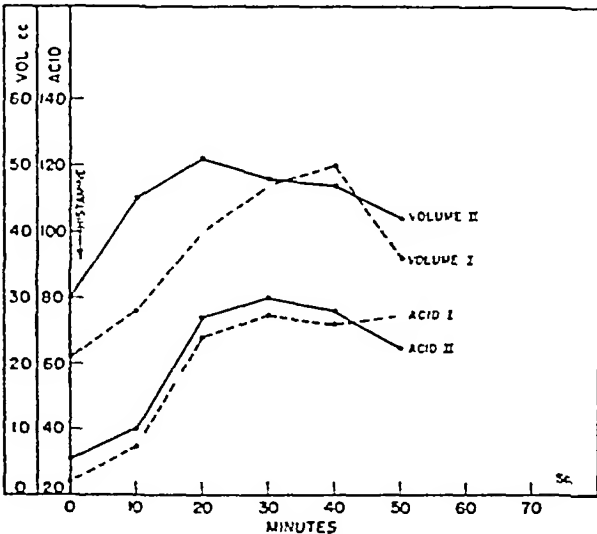


FIG 3 OBSERVATIONS IN CASE 3

(age 52) The three tests are shown in Figure 4. The curves for volumes of secretion are practically unchanged but there is a distinct fall in the degree of acidity, the highest total acidity attained in the three tests being 150, 139, and 125. The man's health has been good throughout the ten year period and there are no digestive symptoms.

Case 5 (Number 180899) Mo a 41-year-old male with thoroughly treated latent syphilis never had evidence of disease of the stomach. He was tested on October 15, 1927, October 5, 1933, and April 21, 1939. Careful re-examination at the time of the last test (age 52) again showed no signs of stomach disease. The results of the three examinations (Figure 5) make it clear that a decline in gastric secretion has taken place especially in the last five years. Evidence may be summarized as follows:

	Highest 10 minute volume	Highest total acid	Total volume of secretion (50 minutes)
	cubic centimeters		cubic centimeters
Test 1	27	131	96.5
Test 2	17	134	53.0
Test 3	11	60	32.5

As far as we know this is the first case in the literature in which a decline of gastric secretion over a long period has been accurately measured.

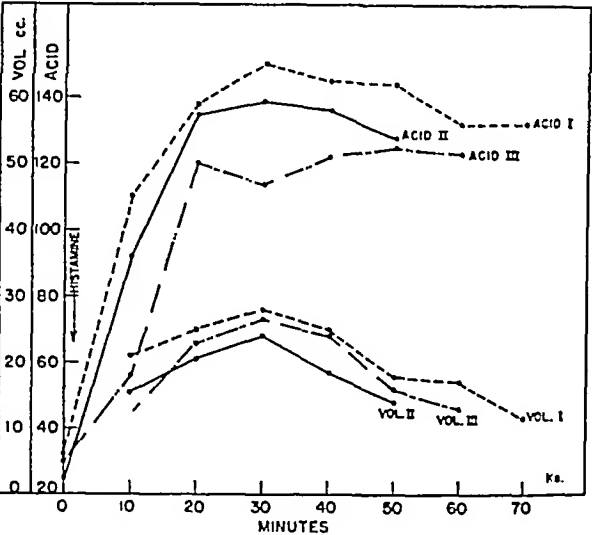


FIG 4 OBSERVATIONS IN CASE 4

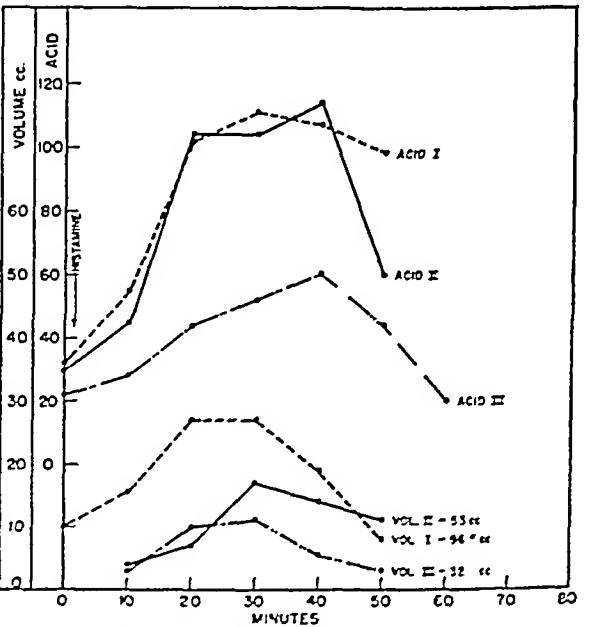


FIG 5 OBSERVATIONS IN CASE 5

In summary, then among five subjects re-examined after intervals of over ten years, three showed practically identical gastric secretion one showed slight decline of acidity but not of volume of secretion, and in one there had been a definite decline both of volume and of acid. The average fall in secretion of large groups of people must then be the resultant of various types of change in different individuals. Just why some normal people preserve their gastric secretion unaltered over many years while others show a rapid decline is entirely obscure. The problem can perhaps be solved by long time studies such as ours controlled by gastroscopic observations. It would be important to know whether any visible changes in the mucosa which can be correlated with alterations in secretion take place.

Heretofore all of the studies on fall of secretion with advancing years have been made by means of histamine tests or with a test meal. It seems of importance to know also if there is a decline of spontaneous basal secretion since the older find-

ings might be due simply to lessened ability to react to secretory stimuli. Recently we have studied the continuous basal secretion of some 75 essentially normal people. The procedure consists in passing a small tube, without any test meal or other stimulus, and collecting the secretions over successive ten minute periods until the rate is constant. The secretion at this point is considered to represent the continuous basal activity of the stomach and with gastric juice of this sort a decline in acidity is also manifest in the older group (Figure 6). The average values for total acidity at ages 20 to 40, 40 to 60 and over 60 were 54, 46, and 34. No cases of true anacidity were included in this series. This fall in basal secretion seems of particular interest since it shows that a disturbance more profound than a mere flagging of response to artificial stimuli underlies defection of secretion.

SUMMARY

Histamine tests repeated in the same individuals after periods of ten or more years show little or no change in some people whereas in others there is a marked fall in gastric secretion. The average curve of decline of gastric secretion with advancing years obtained by examination of large groups of people is a resultant of various findings of this sort. A decline of basal gastric secretion with advancing years has also been demonstrated.

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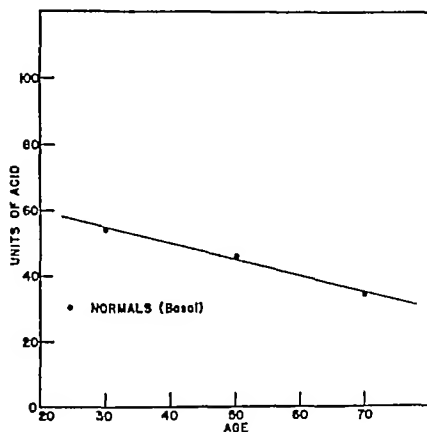


FIG. 6 DECREASE IN BASAL SECRETION OF NORMAL PEOPLE WITH ADVANCING YEARS

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THE STREPTOCOCCAL ANTIFIBRINOLYSIN TEST IN CLINICAL USE¹

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The streptococcal antifibrinolysin test of Tillett and Garner (1) had promise of being a practical serological method for the diagnosis of a recent hemolytic streptococcal infection. The test is simple to perform, inexpensive and uniform in its results. In the experience of Tillett and his associates (2, 3), the test was generally positive in diseases caused by the hemolytic streptococcus, and negative in non streptococcal infections. Subsequent observations by other investigators, however, indicated that the antifibrinolysin test is non specific. Myers, Keefer and Holmes (4) obtained strongly positive tests in 2 of 6 patients with gonococcal arthritis, and in 2 cases of bacterial endocarditis due to the streptococcus viridans. Waaler (5, 6) found the test to be positive in 3 of 4 patients with streptococcus viridans bacteremia and in 6 patients with non streptococcal pneumonia. He concluded that the presence of a positive test is of little diagnostic value.

The antifibrinolysin test has been used as a laboratory aid in our pediatric clinic for over 3 years. Our early experience with the test corroborated the findings of Tillett and associates. Although in very young infants variable results were obtained, we found the test generally to be positive in children recovering from a hemolytic streptococcal infection and negative in other types of infectious disease. Furcolow and Fousek (7) of this clinic reported in 1936 the results of 84 tests on 70 patients. The positive tests numbered 37, and 36 of these were from definite cases of streptococcal infection, the exception was a patient with staphylococcal bacteremia. Of the negative tests none was from cases of hemolytic streptococcal disease. As our series increased, we occasionally encountered positive tests in non-streptococcal infections without an associated bacteremia but we were inclined to view the positive results in these cases as an indication of a recent mild streptococcal infection. In many of the

children there was a history of a recent infection which could have been caused by the hemolytic streptococcus. We did not feel that the rather frequent association of positive tests with a bacteremia interfered with the practical value of the method so long as we were aware that such an exception existed. Such cases were not common and the presence of organisms in the blood stream was easily demonstrated by culture. It did not seem fair to regard the test as non specific on this basis since in the presence of a bacteremia it might be expected that all of the body's methods of defense might be brought into play.

The specificity of the test in pediatric patients first became open to our suspicion during the pneumococcal pneumonia season of 1936 to 1937. Almost all these patients with pneumonia showed strongly positive antifibrinolysin tests at the time of admission to the hospital. In the large majority of these cases there was no history of recent illness, and no clinical or bacteriological evidence of a coincidental hemolytic streptococcal infection. None of the patients received serum. This seemingly definite association of positive antifibrinolysin tests with a specific type of non-streptococcal disease induced us to study the behavior of the test throughout the course of this disease and in those due to the hemolytic streptococcus. Later all available types of infectious disease were studied in this respect since it became apparent, as the test came into routine use, that its non specificity was not limited to pneumococcal pneumonia.

This report represents a study of the trend of repeated antifibrinolysin tests in 15 normal children, 6 newborns and 203 patients of the pediatric age-group.²

METHODS OF STUDY

During a period of over a year repeated antifibrinolysin tests were performed on all patients

¹ Presented in part at the 1938 meeting of The Society for Pediatric Research.

² Children up to 16 years of age are admitted to the pediatric service.

on the pediatric service of New Haven Hospital with definite hemolytic streptococcal infections and with various types of non-streptococcal disease. The non-streptococcal disease group of patients was made up of those in whom there was no evidence, clinical or bacteriological, of recent contact with the hemolytic streptococcus. Included in the study were 15 normal infants and children, and 6 newborns.

Blood for testing was taken at the time of the patient's admission and at 2- to 4-day intervals throughout the period of hospitalization. In a few of the patients, where convalescence did not seem complete at the time of discharge, samples of blood were taken upon their return to the out-patient clinic for follow-up examinations. Each of the 15 normal children was tested at 3- and 4-day intervals during the first week of observation, and then at weekly intervals for a month. Studies on this group covered all seasons of the year. Samples of blood were obtained from the 6 newborns during the first twenty-four hours of life, at the end of 1 week, and on the tenth day of life (time of discharge). Tests were repeated on 3 of the 6 at the time of the routine 6 weeks' clinic check-up.

Antifibrinolysin test. The method is that of Tillett and Garner (1) and is based upon their observation that human hemolytic streptococci produce a substance (fibrinolysin) which will dissolve the plasma-clot of normal individuals, and that the blood of patients recovering from a hemolytic streptococcal infection generally shows resistance (antifibrinolysin) to this lytic action of the organism.

Collection of blood. Three cc. of blood to be tested was obtained by venipuncture and placed in a sterile 110 X 100 mm glass tube containing 60 milligrams of potassium oxalate (0.3 cc. of a 2 per cent solution of oxalate in distilled water). The tube was corked and inverted several times to prevent clotting. The tube was then placed in the ice box until used for the test, which was always done within 48 hours after the blood was taken.

Source of fibrinolysin. Ro, a strain of hemolytic streptococcus isolated in this laboratory from the blood of a child with a fatal septicemia, was used for all the tests. The culture was maintained by daily transfers in beef heart infusion broth containing 0.05 per cent dextrose. This strain has been used daily for about 2 years and regularly lyses the plasma-clot of a normal individual in 15 minutes or less. The fresh culture was routinely tested at about weekly intervals for maintenance of fibrinolytic activity. No variation great enough to affect the test was observed. In our earlier work, duplicate tests were run using a strain of hemolytic streptococcus from a case of scarlet fever as a control. The results were so uniform, however, that for simplicity only strain Ro was used in this study. From time to time we have also employed the patient's own strain of hemo-

lytic streptococcus as a source of fibrinolysin for the test. Again, there was no significant difference between the results with Ro and with the homologous strain.

Description of test. The oxalated sample of blood was centrifuged for 3 to 4 minutes at about 1500 r.p.m., and 0.2 cc. of plasma was removed and diluted with 0.8 cc. of sterile physiological salt solution. To this was added 0.5 cc. of an 18-hour broth culture of the hemolytic streptococcus. Lastly, 0.25 cc. of calcium chloride (0.25 per cent solution in normal salt solution) was added, and the thoroughly mixed contents of the tube were immediately incubated in a water bath at 37.5° C. In about 10 minutes a solid opaque clot was formed. The interval between the time of clotting and that of complete liquefaction was taken to indicate the degree of resistance of the patient's plasma to the fibrinolytic action of the hemolytic streptococcus. This resistance (amount of antifibrinolysin) was graded as follows:

- 4+ indicates no lysis of the plasma-clot in 24 hours
- 3+ indicates complete lysis in 8 to 24 hours
- 2+ indicates complete lysis in 3 to 8 hours
- 1+ indicates complete lysis in 1 to 3 hours
- indicates complete lysis in less than 1 hour

In the text, a positive test refers to a 1+ to 4+ reaction. Tests were terminated at 24 hours since clots which remained solid for this length of time continued so for days.

Bacteriological studies. Fresh beef heart infusion medium is used routinely in the pediatric bacteriological laboratory. Cultures of the nose, throat, blood, and material from purulent lesions (spinal fluid, pleural fluid, aural discharge, mastoid pus, etc.) were taken at the time of the patient's admission and subsequently as seemed indicated. Colonies from any of the above sources which resembled those of the hemolytic streptococcus were tested for bile insolubility and production of a soluble hemolysin for rabbit's erythrocytes, and were then grouped by the serological method of Lancefield (8, 9, 10).

RESULTS

Early in the study it became apparent that the trend of repeated antifibrinolysin tests in pneumococcal pneumonia was different from that generally seen in hemolytic streptococcal infections. In streptococcal diseases the tests were generally negative during the acute stage of the illness and became increasingly positive at about the time of recovery. In pneumococcal pneumonia the strongly positive tests, which were commonly encountered, were present only during the acute febrile stage of the infection and subsequent tests showed a rapid decrease to negative. To demonstrate this difference in the trend of the tests, charts of temperature course and antifibrinolysin

test results accompanied by a brief clinical summary are given for a typical case of pneumococcal pneumonia (Chart 1) and of a hemolytic streptococcal infection, scarlet fever (Chart 2). Both patients were of the same age and were hospitalized on the same day of illness.

As the study was enlarged to include many of the common types of non streptococcal infection and a larger group of hemolytic streptococcal diseases, a total of 6 antifibrinolysin test trends was observed.

- A Negative tests which persisted throughout the patient's illness and convalescence (Chart 3)
- B Positive tests during the acute febrile stage of an infection which rapidly changed to negative ones (Chart 1)
- C Negative tests during the acute stage of the disease which became increasingly positive during the period of convalescence (Chart 2)
- D Strongly positive tests which persisted throughout the patient's illness and convalescence (Chart 4)

E Positive tests during the acute illness which changed to negative and then reverted to positive (Chart 5)

F Negative tests which rapidly changed to positive and then back to negative (Chart 6)

For simplicity these 6 trends are represented graphically in Chart 7 and will subsequently be referred to by letter and a descriptive phrase, e.g. Trend A (persistently negative). When the results are plotted it becomes more than ever apparent that there is a definite trend in the course of the tests and that the trend is a smooth one without gross fluctuations.

The 6 antifibrinolysin trends and the conditions in which they were found are summarized in Chart 8.

The group of "normal" individuals consisted of 5 infants and 10 children who were apparently in good health and free from any type of infection. These subjects varied from 3 months to 16 years of age. The 6 healthy newborns who were included in the study are tabulated separately since their antifibrinolysin trend was found to be distinctly different from that of the older infant.

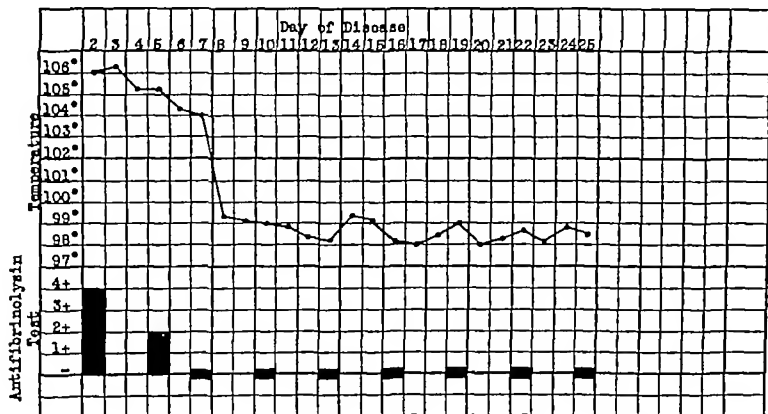


CHART 1 PATIENT G J AGE 3 YEARS. DIAGNOSIS PNEUMOCOCCAL PNEUMONIA, TYPE I

Child was admitted on the second day of the disease with physical and roentgenological signs of right lower lobe pneumonia. Pneumococcus type I was the predominating organism in the nose and throat cultures; there were no hemolytic streptococci. Child remained very ill until the seventh day when his temperature dropped to normal by crisis. His subsequent course was satisfactory.

Antifibrinolysin tests. Test was strongly positive on admission. It was still positive on the fifth day of the disease but to a lesser degree. With the fall in temperature on the seventh day it became negative. Subsequent tests were also negative.

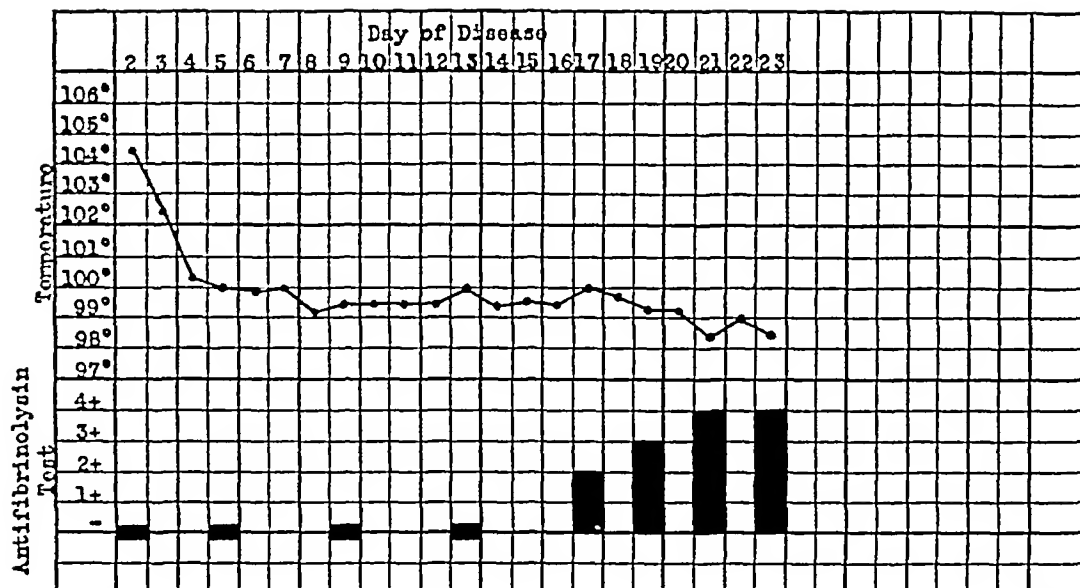


CHART 2. PATIENT M D AGE 3 YEARS DIAGNOSIS SCARLET FEVER

Child was admitted on the second day of illness with classical signs of scarlet fever of moderate severity. Throat culture showed a predominance of hemolytic streptococci. Child's temperature dropped rapidly toward normal, recovery was uneventful and desquamation occurred.

Antifibrinolysin tests Tests were negative throughout the active stage of the disease and became increasingly positive during convalescence.

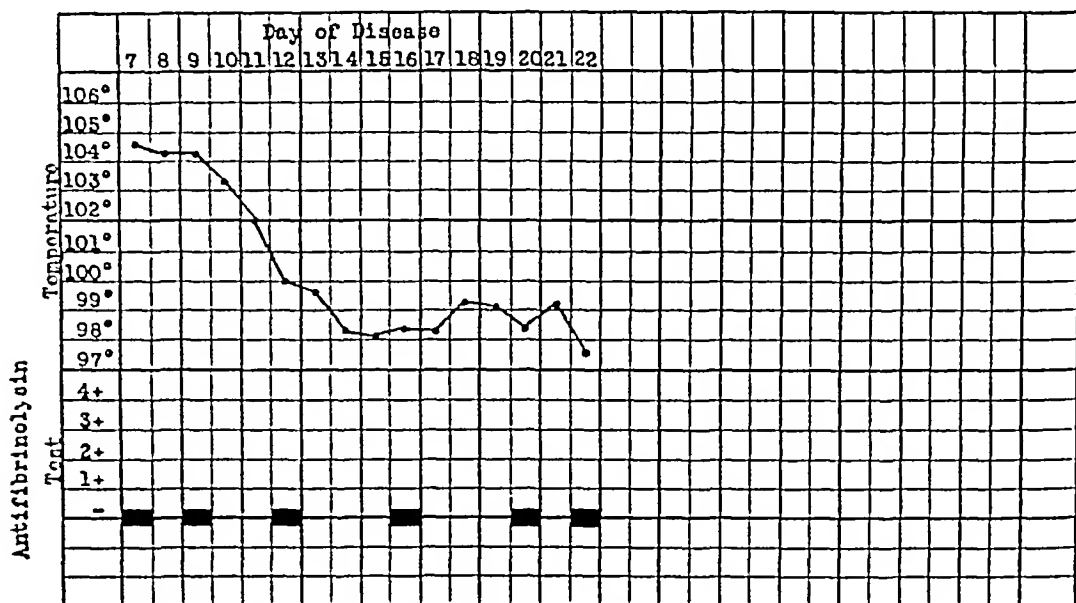


CHART 3. PATIENT M M AGE 3 YEARS DIAGNOSIS PYELITIS (B COLI)

Child was admitted on the seventh day of the disease with a history of high fever, urinary frequency, and abdominal pain. Physical examination was negative except for costovertebral angle tenderness on the right side. Urine showed a 3 plus albumin and numerous white blood cells. B coli was cultured from the urine. Nose and throat cultures were negative for hemolytic streptococci. Under sulfanilamide therapy child's temperature dropped to normal on the thirteenth day and remained within normal limits. The urine cleared coincidentally with the fall in temperature.

Antifibrinolysin tests Tests were persistently negative throughout the child's illness and convalescence.

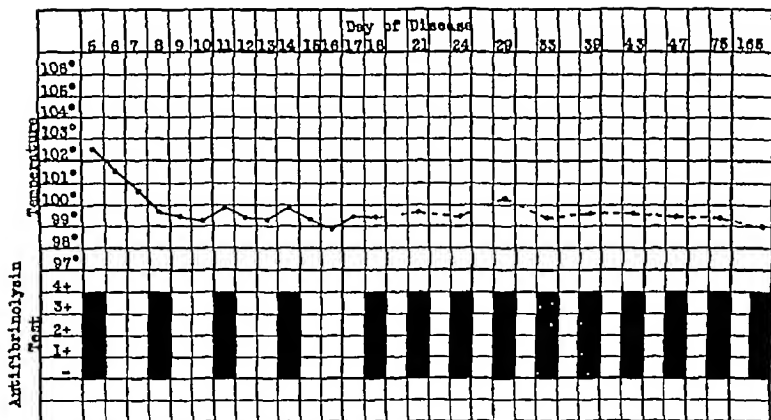


CHART 4 PATIENT A. S. AGE 13 YEARS. DIAGNOSIS RHEUMATIC FEVER, RHEUMATIC HEART DISEASE

Child was admitted on the fifth day of the disease with fever, epistaxes and pains in her knees. The heart was overactive and the knees were swollen and tender. The sedimentation rate was 26 mm. per hour. Nose and throat cultures showed a predominance of hemolytic streptococci until the twenty-seventh day of illness. Subsequent cultures were negative. Signs of rheumatic activity persisted until the thirty-ninth day. Child was transferred on the forty-seventh day to the Children's Community Center for convalescence. In the meantime, signs of a mitral and possibly an aortic heart lesion had appeared.

Antifibrinolysin tests. Tests were persistently positive during the entire period of hospitalization. Repeat tests 1 and 4 months after child's discharge were still strongly positive although her convalescence was uncomplicated.

There were 75 cases of hemolytic streptococcal infection. Seventy of these were primary infections and include scarlet fever (25 cases), erysipelas (7 cases), acute tonsillitis and cervical adenitis (25 cases), otitis media and mastoiditis (10 cases), pneumonia (1 case), meningitis (1 case), and cellulitis (1 case). In 3 cases the hemolytic streptococcal infection followed a non-streptococcal disease: tuberculous pneumonia later complicated by hemolytic streptococcal tonsillitis and purulent otitis media which was followed by nephritis (1 case), pneumococcal pneumonia followed during the period of convalescence by hemolytic streptococcal tonsillitis and later acute hemorrhagic nephritis (1 case), and *B. coli* infection of the urinary tract complicated by hemolytic streptococcal tonsillitis and cervical adenitis (1 case). Both of the remaining 2 patients appeared from their course to be straightforward cases of scarlet fever. However the presence of patho-

genic pneumococci—type VII in one instance and type XIV in the other—in the nose and throat of these 2 patients suggests the possibility of a recent or simultaneous pneumococcal infection.

Thirty cases of acute rheumatic fever, 3 of chorea and 11 of acute hemorrhagic nephritis were studied in view of the possible or probable relationship of the hemolytic streptococcus to these diseases.

In the non streptococcal disease group, upper respiratory infections (6 cases), influenza (5 cases), pneumococcal pneumonia (32 cases) and poliomyelitis (7 cases) are tabulated separately as they were the most common types of non streptococcal infection encountered during the study. There were 34 patients with other varieties of non streptococcal disease. Of these 20 are classified as "mild" and 14 as "severe," depending on the severity of the illness in the particular patient. The "mild" infections include pertussis

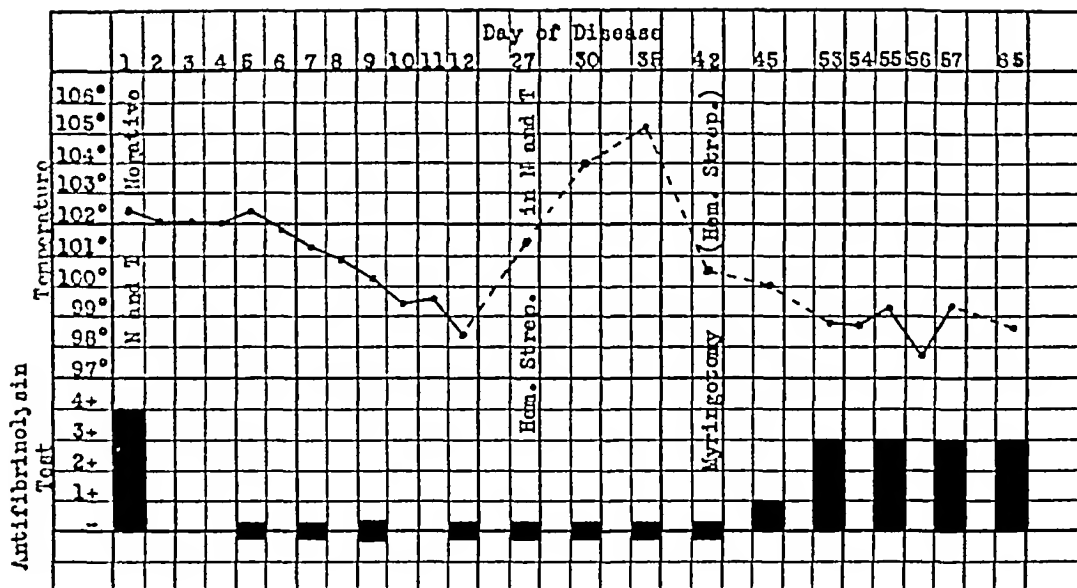


CHART 5 PATIENT T U AGE 14 MONTHS DIAGNOSIS TUBERCULOUS PNEUMONIA WITH EFFUSION, ACUTE PHARYNGITIS, CERVICAL ADENITIS AND BILATERAL PURULENT OTITIS MEDIA (HEMOLYTIC STREPTOCOCCAL), ACUTE HEMORRHAGIC NEPHRITIS

Baby was admitted on apparently the first day of illness with physical and roentgenological signs of pulmonary tuberculosis with pleural effusion on the right side. Tuberculin test was strongly positive. Parents and 4 siblings had active tuberculosis. A thoracentesis yielded sterile straw-colored fluid. On the twenty-seventh day, the baby developed profuse nasal discharge, fiery red throat, and enlarged cervical glands. Nose and throat cultures, which were previously negative for pathogens, showed a predominance of hemolytic streptococci. On the forty-second day the right ear began to discharge and a left myringotomy was performed. Hemolytic streptococci were cultured from the pus from both ears. Urine at that time showed albumin and red blood cells. The streptococcal infection and the nephritis gradually subsided. The baby was discharged to a sanatorium because of persistence of the tuberculous process.

Antifibrinolysin tests Test was strongly positive during the acute phase of the tuberculous pneumonia but rapidly became negative. Tests remained negative during the acute febrile stage of the hemolytic streptococcal infection but became strongly positive during convalescence.

(3 cases), bronchitis (4 cases), chicken pox (1 case), mumps (1 case), bronchopneumonia (5 cases), urinary tract infection (2 cases), diphtheria (1 case), gonococcal vaginitis (1 case), measles (1 case), and exanthem subitum (1 case). The "severe" infections consist of staphylococcal septicemia (1 case), typhoid fever (3 cases), bacillary dysentery (2 cases), meningococcus meningitis (2 cases), pulmonary tuberculosis (1 case), pyelitis due to *B. coli* (2 cases), encephalitis (1 case), pneumococcal empyema (1 case), and streptococcus viridans septicemia (1 case).

Antifibrinolysin Trend A (persistently negative) was present in all of the 15 normal infants and children. It was also found in all of the cases of mild and in some of the cases of severe

non-streptococcal infection. It occurred occasionally in a hemolytic streptococcal illness and in acute rheumatic fever, chorea and acute nephritis.

Trend B (positive to negative) was frequently observed in the more severe non-streptococcal diseases, notably pneumococcal pneumonia, in all the newborns, and occasionally in patients with a hemolytic streptococcal infection.

Trend C (negative to positive) occurred in 53 of the 70 cases of primary hemolytic streptococcal disease, and was seen in no other condition.

Trend D (persistently positive) was found in most of the cases of rheumatic fever, chorea and acute nephritis, in a few cases of hemolytic streptococcal disease, and in 1 patient with typhoid fever. Unfortunately, this patient was not fol-

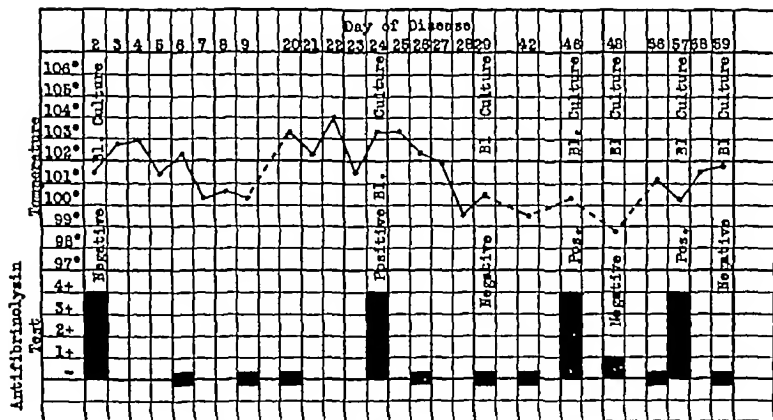


CHART 6 PATIENT R. S. AGE 11 YEARS. DIAGNOSIS CONGENITAL HEART DISEASE WITH SUBACUTE BACTERIAL ENDOCARDITIS (STREPTOCOCCUS VIRIDANS)

Child, known to have congenital heart disease was admitted on the second day of his acute illness with cough bloody sputum, chills and high fever. Signs on physical and roentgenological examination were those of congenital heart disease with pulmonary infarction. There were no petechiae seen and admission blood culture was negative. Repeated nose and throat cultures showed no hemolytic streptococci. On the twenty fourth day of illness streptococcus viridans was cultured from the blood stream. Child continued to have bouts of fever associated with petechiae and positive blood cultures through out his seven months period of hospitalization and course was gradually downhill. Postmortem examination verified the clinical diagnosis.

Antifibrinolysin tests The test was strongly positive on admission and became negative in a few days. With each shower of bacteria into the blood stream the test rapidly became positive and returned to negative when blood cultures showed no growth.

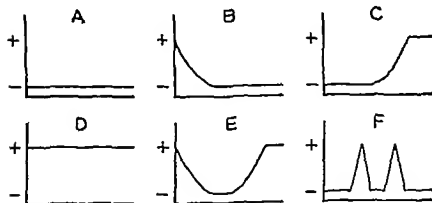


CHART 7 TRENDS OF ANTIFIBRINOLYSIN TESTS

Trend A persistently negative tests Trend B positive to negative tests Trend C, negative to positive tests Trend D persistently positive tests Trend E positive to negative to positive tests Trend F negative to positive to negative tests (spiking)

lowed through convalescence it is possible that if the period of study had been longer the trend would have been B rather than D

Trend E (positive to negative to positive) ap-

peared in 3 cases of non streptococcal infection followed during convalescence by a streptococcal illness and in the 2 cases in which the bacteriological findings suggested a possible simultaneous pneumococcal and hemolytic streptococcal infection

Trend F (spiking) was encountered in only 1 instance a case of congenital heart disease with streptococcus viridans endocarditis

DISCUSSION

A study of the trend of the streptococcal antifibrinolysin test under various conditions of health and disease in individuals of the pediatric age-group demonstrates several interesting points

The test does not seem to be influenced by minor variations in the child which may occur either in health or in the presence of a mild infection Tests were repeatedly negative in the







Conditions studied	Trends of antifibrinolysin test						
	4+	A	B	C	D	E	F
	—						
Normal	15						
Scarlet fever and other hemolytic streptococcus infections	8	4	53	5	(5)		
Rheumatic fever and chorea	3			30			
Acute nephritis	1			10			
Non streptococcal diseases							
Upper respiratory infections	6						
Influenza	2	3					
Pneumococcal pneumonia	4	28					
Poliomyelitis	4	3					
Other "mild" infections	20						
Other "severe" infections	3	9		1			1
Newborns		6					

CHART 8 TRENDS OF ANTIFIBRINOLYSIN TESTS AND CONDITIONS UNDER WHICH THEY WERE OBSERVED

Note: Figures represent the number of cases, figure in parenthesis refers to cases of secondary, or possible secondary, hemolytic streptococcal infection

normal infants and children reported, and single tests on a much larger series of healthy young individuals and of children with various types of non-infectious diseases have corroborated this finding. Consistently negative tests were also found in pediatric patients with mild non-streptococcal infections.

Newborn infants, however, furnished an exception to this rule. Strongly positive tests were obtained with venous blood of all 6 newborns and with their respective placental blood, in spite of the fact that tests on the mothers' venous blood, just prior to and just after delivery, were negative. In none of the mothers was there any indication of a recent hemolytic streptococcal infection. In 3 of the babies, the test had become negative by the tenth day of life, while in the remaining 3, tests were still positive on the tenth day, but were negative at 6 weeks of age. Lippard and Wheeler (11) obtained maximally positive tests in 14 of the 28 newborns whom they studied. It would be of much interest to ascertain if this is a true streptococcal antifibrinolysin which has been stored up in the fetus.

Positive tests were obtained in 67 of the 75 patients with hemolytic streptococcal infections at

some time during the course of their illness and convalescence. In 53 of the 70 patients with primary hemolytic streptococcal infections, the test was negative during the acute phase of the disease and became positive about the time of recovery. This trend, Trend C, (negative to positive), which was present in over 70 per cent of the cases studied, was seen in no other type of infection. Trend E (positive to negative to positive), which was observed in 3 patients with secondary and 2 patients with probable secondary hemolytic streptococcal infections, appears to represent "hemolytic streptococcal." Trend C (negative to positive) preceded by "non-specific" Trend B (positive to negative), such as might be expected in a hemolytic streptococcal infection complicating a non-streptococcal illness. Of the 8 patients in whom repeated tests were negative, 2 were infants, 2 were children who had a prolonged illness with complications, and 1 was an infant with a fatal infection. Tillett (3) found that in patients with prolonged or fatal streptococcal infections the test was apt to remain negative or to become only weakly positive, and Lippard and Johnson (12) report that the antibody response in infancy as determined by the

antifibrinolysin and antistreptolysin content of the blood may be poorly developed

Concerning the non specific aspect of the antifibrinolysin test, there would seem to be little doubt that the more severe types of non streptococcal infection are also capable of producing a positive test in children. Positive tests are common during the acute febrile stage of such diseases. It is of much interest, however that the trend of the results was strikingly different from that seen in the majority of patients with hemolytic streptococcal infections. The mechanism for this production of positive tests in non streptococcal infections is not apparent at this time. It might find an analogy in the increase in the streptococidal power of the blood of patients in the active phase of infections due to a variety of organisms as described by Tillett (13). It should be noted that Friedemann and Sutcliffe (14) found an unusual polysaccharide in the blood in similar conditions.

The presence of positive tests in rheumatic fever, chorea, and nephritis is in accord with the findings reported in the literature (3, 4, 5, 6, 12, 15, 16, 17, 18). Six of our patients with rheumatic fever were retested 3 to 5 months after discharge from the hospital and all continued to show strongly positive tests although they had apparently been free from any significant infections. The positive tests seen during convalescence from uncomplicated hemolytic streptococcal infections such as scarlet fever were found to persist for several days or at the most a few weeks. This strong association of positive tests with rheumatic fever suggests that the antifibrinolysin test may be of diagnostic aid. Unfortunately, the presence or absence of an etiological relationship between the hemolytic streptococcus and rheumatic fever cannot be determined from the trend of the antifibrinolysin tests. The trend of the test in rheumatic fever is neither of the "non specific" type, Trend B (positive to negative) nor of the "hemolytic streptococcal" type, Trend C (negative to positive). There are three points however which are consistent with such a relationship. (1) If rheumatic fever is a sequel to infection by the hemolytic streptococcus it would be anticipated that only the latter portion—the positive phase—of Trend C would be obtained. (2) the incidence of persistently positive

tests (10 out of 11 patients) in this disease is remarkably similar to that observed in the cases of acute nephritis, all of which followed scarlet fever or other types of hemolytic streptococcal infection (3) with the possible exception of one patient with typhoid fever who was not followed after discharge from the hospital in no disease other than rheumatic fever did the duration of positive tests at all approach that seen in nephritis.

SUMMARY AND CONCLUSIONS

The trends of repeated streptococcal antifibrinolysin tests in 15 normal infants and children, 6 newborns, and 203 patients of the pediatric age group are reported.

The study suggests that, although a single test may be of no diagnostic value in the pediatric laboratory unless viewed in the light of the history and bacteriological findings the trend of several tests may be significant. The type of trend that was observed in the majority of patients with hemolytic streptococcal infections was found in no other type of disease.

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THE ANTISTREPTOLYSIN TITER IN RHEUMATIC FEVER, ARTHRITIS AND OTHER DISEASES

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Since 1933 a number of serologic tests have been made in the study of patients with rheumatic fever, arthritis, and several other diseases who have come under the authors' care. These tests include gonococcus complement fixation, hemolytic streptococcus agglutinin and precipitin tests and antifibrinolysin and antistreptolysin titrations. A preliminary report of the results of these was made in 1936 (1). The purpose of the present report is to give a more detailed analysis of the results of antistreptolysin determinations performed from 1933 to the end of 1938.

We have determined the titer of the serum of normal subjects and patients with the following diseases:

1. Scarlet fever
2. Rheumatic fever and chorea
3. Rheumatoid and other types of arthritis
4. A few cases of such miscellaneous conditions as erythema nodosum, pericarditis, lupus erythematosus disseminatus and periarteritis nodosa

The cases studied were from the Third (New York University) Medical Division of Bellevue Hospital and from the Arthritis Clinics of New York University College of Medicine. The sera were analyzed without knowledge of the clinical diagnoses. The serological determinations were done according to the method of Hodge and Swift (2). Because of the time required for antibody formation to take place, no patient with antistreptolysin titer within the normal range was included in our analysis unless serial determinations were made over a period of at least 4 weeks.¹ In all, 1,539 determinations have been made on the sera of 817 patients with the diseases listed above. We have also collected and rearranged results

obtained by others for the purpose of convenient reference.

I. ANTISTREPTOLYSIN TITER IN NORMAL SUBJECTS

An analysis was made of the sera of 39 normal persons consisting of medical students and physicians. These subjects were free from any upper respiratory infection or other illness for a minimum of 4 weeks prior to examination. The titer was 25 units or less in 21, 50 units in 12, 100 units in 5, and 150 units in 1 person.

Table I summarizes these results and those of other investigators. The median titer reported by most workers is 100 units or less. Myers and Keefer found the average (not median) titer to be 213 units. They used sheep erythrocytes instead of the customary rabbit cells; however, and von Hellens (3) had observed that sheep cells were more resistant to hemolysis by streptolysin than rabbit erythrocytes.

In view of our results we have considered 100 units per cc. of serum as the upper limit of normal. However, a rise in titer in serial tests, even though not exceeding 100 units, suggests hemolytic streptococcus infection as is shown below in tests performed on patients with scarlet fever.

II. ANTISTREPTOLYSIN TITER IN SCARLET FEVER

In 1934 we studied 21 cases of scarlet fever admitted to Willard Parker Hospital during the months of January and February of that year. Blood was collected from each patient on the day of admission and again upon discharge.* As will be seen from Table II, all patients except 1 had a normal antistreptolysin titer on admission. All but 4 showed an elevation of antistreptolysin titer during the course of the disease, although only 10

*The authors are indebted to Dr. Jesse M. Bullowa and Dr. I. H. Scheffer for permission to obtain these sera.

¹Except in the scarlet fever patients, some of whom were followed only 3 weeks.

Todd (4), Griffiths (7) Longcope (8) and Blair and Hallman (9) report an elevated titer in 85 per cent to 100 per cent of patients with active rheumatic fever. In most of these reports no mention is made of the presence or absence of preceding respiratory infections or the results of throat cultures.

We have studied 171 adults with active rheumatic fever and 139 with inactive rheumatic heart disease. The patients were under observation on the wards and in the clinics for a period ranging from 4 weeks to several years. The criteria for activity were (1) history of palpitation, precordial pain, fever, joint pains, or undue weight loss, (2) fever, tachycardia, gallop changing murmurs or rhythm, pericardial friction rub, rheumatic skin manifestations or subcutaneous nodules, (3) significant electrocardiographic changes, (4) elevated erythrocyte sedimentation rate or leukocytosis.

In Table IV we have divided the cases into an active and inactive group and have subdivided each group according to the presence or absence of an upper respiratory infection ('cold,' sore throat, pharyngitis, tonsillitis or bronchitis) within 4 weeks of serological studies. The patients having active disease have been further classified in relation to the presence of polyarthritis, carditis, or both. It is clear from this study that the median antistreptolysin titer of the patients with active rheumatic fever was above normal, while

subjects observed for several months during comparatively good health.

this was not true of the patients with inactive rheumatic heart disease. In the patients with manifestations of active rheumatic fever, the antistreptolysin titers were above 100 units in 76 per cent to 83 per cent of those who gave a history of preceding upper respiratory infection as compared with 54 per cent to 68 per cent of those without such a history (Figure 1). There was no significant difference between the patients who had rheumatic polyarthritis without carditis or

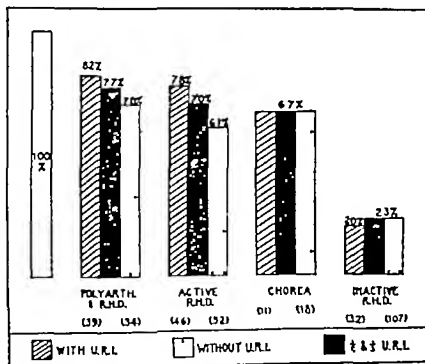


FIG. 1. ELEVATED ANTISTREPTOLYSIN TITER IN 339 RHEUMATIC SUBJECTS IN RELATION TO UPPER RESPIRATORY INFECTION

U. R. I. = upper respiratory infection
 & = with and without
 Polyarthr. = polyarthritis
 R. H. D. = rheumatic heart disease.

TABLE IV

Maximum antistreptolysin titer (units per cc) in adult rheumatic subjects

	Active rheumatic fever						Inactive rheumatic heart disease	
	Polyarthritis without obvious carditis		Active carditis with polyarthritis		Active carditis without polyarthritis			
	With upper respiratory infection	Without upper respiratory infection	With upper respiratory infection	Without upper respiratory infection	With upper respiratory infection	Without upper respiratory infection	With upper respiratory infection	Without upper respiratory infection
Number of patients	39	34	34	28	12	24	35	104
Average number of bleedings	1.9	1.6	2.8	2.0	2.5	1.7	2.0	2.0
Median titers	250	225	200	200	200	150	100	100
Per cent of patients above 100 units	82	68	76	68	83	54	20	23
Number of patients above 100 units	32	33	26	19	10	13	7	24

those who had carditis without polyarthritis or those who had both polyarthritis and carditis. We were unable to find a correlation between the severity of the disease and the degree of titer elevation. This finding does not agree with the experience of Coburn and Pauli who reported distinctly higher titers in patients with intense or prolonged rheumatic activity than in patients whose rheumatism was of brief duration or mild character. Of the 139 patients with inactive rheumatic heart disease, 35 gave a history of a recent upper respiratory infection and 104 did not. The per cent that showed an elevated titer in these two groups was practically the same, about 20 per cent.

B Chorea

Although we believe Sydenham's chorea should be considered a manifestation of rheumatic fever, we have analyzed our data in these cases separately in view of recent reports questioning this relationship (10). The sera of 29 patients with chorea have been studied.⁴ In most instances there was no evidence of organic heart disease and no history of polyarthritis or previous upper respiratory infections. The ages varied from 5 to 21 years.

Sixty-seven per cent of the patients with or without a preceding upper respiratory infection showed an antistreptolysin titer above 100 units.⁵ The results with patients having chorea are comparable to the incidence of elevated titers in the group of patients with active rheumatic carditis and polyarthritis discussed above.

Twenty patients were treated with fever therapy, the hyperpyrexia being induced either by repeated typhoid vaccine injections or by means of the hypertherm. As has been previously noted by Todd (4) and by Coburn and Pauli (5), typhoid vaccine injections appeared to have no influence on the antistreptolysin titer. Similarly, fever induced by the hypertherm seemed to have no effect on the titer.

We could find no strict correlation between the

age of the patient, the severity of the chorea, and the height of antistreptolysin titer.

IV ANTISTREPTOLYSIN TITER IN ARTHRITIS

A Rheumatoid arthritis

Blair and Hallman (9) found that of 45 patients with rheumatoid arthritis, 15 showed a high antistreptolysin titer. Longcope (8) observed that the antibody level was raised in 25 of 55 cases. Dawson and Olmstead (11) studied 219 cases and concluded that in the great majority of those whose illness was of long duration the titer was within normal limits, whereas in the cases of recent and particularly of acute onset, a significant number showed an abnormal titer.

We determined both the agglutinin titer and antistreptolysin titer of 205 sera from 72 patients with rheumatoid arthritis. The same strain of hemolytic streptococcus was used as an antigen for testing the content of both antibodies.⁶ Record was made of any history of a preceding upper respiratory infection and the duration of the disease. As will be seen from Table V, 85 per cent of the patients showed a normal titer and the median was 50 units. Of the 13 patients who had suffered with this condition for less than one year, 9 or 69 per cent gave normal titers, of the remaining 4 with titers above 100 units, 3 reported a recent upper respiratory infection. Of the 11 patients in the entire series with elevated antistreptolysin values, 36 per cent gave a history of recent respiratory infection, whereas only 10 per cent of patients with normal titers presented such a history. Thus, in most of our cases of rheumatoid arthritis, the antistreptolysin titer was within normal limits regardless of the stage of the disease or the severity of clinical symptoms. We found no correlation between the antistreptolysin and the hemolytic streptococcus agglutinin titers, three-quarters of the patients with high agglutinin titers (1:80 to 1:640) had a normal antistreptolysin titer.

B Other types of arthritis

Antistreptolysin determinations were made in 9 types of arthritis besides rheumatic fever and

⁴ We are grateful to Dr. Charles H. Smith and to the late Dr. Lucy P. Sutton for permitting us to study 18 cases from the Children's Medical Service of Bellevue Hospital.

⁵ Longcope (8) reports that of 8 cases of chorea 7 showed a level above 100 units.

⁶ We used N.Y. 5 as the antigen for the agglutinin titer and WPRL for the production of streptolysin. We subsequently learned that these two labels refer to the same strain.

TABLE V

*Antistreptolysin titer in 72 patients with rheumatoid arthritis and its relation to hemolytic streptococcus agglutinin titer upper respiratory infections and duration of illness**

Titer	Number of patients	Number of determinations	Early stages less than one year	Late stages, more than one year†	Preceding upper respiratory infection	Hemolytic streptococcus agglutinin titer			
						Negative	1:20 or 1:40	1:80 or 1:160	1:320 or 1:640
units per cc.									
Less than 25	8	8	0	5	1	1	2	1	1
25	12	15	4	15	2	0	0	6	6
50	24	27	4	15	2	0	0	11	9
100	20	23	17	2	2	1	1	4	8
150	2	4	1	0	2	1	0	0	1
200	3	4	1	2	1	0	0	1	2
250	4	21	2	2	1	0	1	0	2
320	1	9	0	1	0	1	0	0	0
400	1	12	0	1	1	0	0	0	1

* Where more than one determination was done in a given case only the maximum titer was considered.

† In 9 of the 72 cases the exact duration of the illness was not determined. Of these the titer was 25 units in 3, 50 units in 3, 100 units in 2 and 150 units in 1.

rheumatoid arthritis. The criteria for classifying these types have been described elsewhere (12). The bases for differentiation were purposely made very strict and all cases about which we had any doubt were listed in one of the two "unclassified" categories. The results are presented in Table VI. We studied 493 serum specimens of 320 patients. Eighty five per cent of the entire group

had titers within normal limits. Of the 15 per cent showing a high antibody level, at least one fourth were known to have had a recent upper respiratory infection. The highest percentage of elevated titers was found in the patients with unclassified types of arthritis, some of whom probably had atypical rheumatic fever. It should be noted that 2 patients with suppurative arthritis due to hemolytic streptococcus showed abnormal titers.

Table VII summarizes the titers observed in all

TABLE VII

Summary of the antistreptolysin titer (units per cc.) of all cases of arthritis studied

Type of arthritis	Number of patients	Median titer	Per cent of patients with titers above 100
Rheumatic poly arthritis	135	250	74
Rheumatoid arthritis	72	50	15
All other types of arthritis*	318†	50	15

* It should be noted that in this group have been included the unclassified types of arthritis, some of which may have been atypical rheumatic or rheumatoid arthritis. These unclassified types have been listed separately in Table VI.

† The 2 cases of hemolytic streptococcus arthritis have not been included.

TABLE VI

*Antistreptolysin titer (units per cc.) in various types of arthritis**

Type of arthritis	Num-ber of patients	Num-ber of deter-mina-tions	Median titer	Per cent above 100	Number of patients with the following titers:										Upper respiratory infection
					Less than 25	25	50	100	150	200	250	300	Above 300		
			units per cc.	per cent											
Osteo-arthritis	106	148	50	3	6	33	45	19	2	0	1	0	0	1	
Gonococcal	60	100	50	10	2	11	24	17	3	1	0	1	1	3	
Tuberculous	5	5	50	20	0	1	3	0	1	0	0	0	0	0	
Hemolytic strepto-coccal (suppurative)	2	2		100	0	0	0	0	0	1	0	0	1	0	
Meningococcal	2	2		0	0	0	1	1	0	0	0	0	0	0	
Spondylitis															
Ankylopoietica	6	12	100	16	1	0	1	3	1	0	0	0	0	0	
Gout	10	20	50	0	0	3	4	3	0	0	0	0	0	0	
Infectious—type unclassified	95	152	50	29	5	14	28	20	12	6	3	7	0	7	
Wholly unclassified	34	52	50	23	2	7	9	8	4	3	1	0	0	2	
Total	320	493	50	15	16	69	115	71	23	11	5	8	2	13	

* When more than one determination was done only the maximum titer was considered. The figures under the respective titers represent number of patients having such titers. The last vertical column indicates the number of patients with titers above 100 known to have had recent upper respiratory infections.

cases of arthritis studied by us. It is interesting that the incidence of elevated titers in the patients with miscellaneous arthritides is exactly the same as in the group with rheumatoid arthritis. In this series of patients, then, rheumatoid arthritis was no more apt to be accompanied by elevated antistreptolysin titers than were such diseases as gonococcal and tuberculous arthritis. The figures in this table also show that the titer may be used as a diagnostic aid in cases of acute arthritis, when elevated, it is suggestive (although not diagnostic) of rheumatic polyarthritis.

ANTISTREPTOLYSIN TITER IN OTHER CONDITIONS

Erythema nodosum may be encountered in cases of infection with hemolytic streptococcus and in tuberculosis. We have made observations on 6 patients with this skin manifestation. Only 1 patient had tuberculosis, and she had a titer of 50 units. In the remaining 5, the maximum titers were 500, 350, 250, 150, and 50. Conclusions cannot be drawn from such a small series. If additional studies should corroborate these results, antistreptolysin determinations might be a useful aid in separating the cases of erythema nodosum associated with hemolytic streptococcus infection from other types.

Acute pericarditis, with or without effusion, is associated, in most instances, with rheumatic fever or tuberculosis. Clinically, the differential diagnosis in some cases may be very difficult. We have observed 7 cases of acute pericarditis. In 6, this condition developed in the course of rheumatic fever, and every patient showed a titer above 100 (400, 300, 200, 150, 150). The titer in the 1 case of tuberculous origin was 50 units.

Lupus erythematosus disseminatus and periarthritis nodosum. Because of the similarities between some features of these two diseases and rheumatic fever, it was considered of interest to make antistreptolysin determinations in them. Two patients with each disease (confirmed at necropsy in each instance) were followed with serial bleedings over many months up to the time of death. The antistreptolysin titers were well within normal limits at all times.

DISCUSSION

A serologic test such as the antistreptolysin titer can be of interest to the clinician chiefly from

two points of view: (1) the information it may give as to the etiology of certain diseases, and (2) its possible usefulness as a prognostic and diagnostic aid.

All investigators here cited, with the exception of Wilson and her associates, have found an elevated antistreptolysin titer in more than 80 per cent of patients with rheumatic fever. Both Coburn and Todd concluded from this that patients with rheumatic fever are responding immunologically to a hemolytic streptococcus infection. Wilson, Wheeler and Leask (6), on the other hand, believed that in their rheumatic patients "the rise of the level of antistreptolysin in the serum following respiratory infections seemed directly related to the extent of the local and constitutional symptoms irrespective of the presence of hemolytic streptococcus in the pharyngeal flora." They reported also that only one-third of their patients with active rheumatic fever who did not have respiratory infections exhibited a rise in antistreptolysin titer and that the titer of active rheumatic subjects experiencing respiratory infections was similar to that of inactive rheumatic subjects with these infections.

Our results do not bear out these findings of Wilson and her associates, although the antistreptolysin titers did tend to be higher in patients who developed rheumatic fever after distinct respiratory infections than in those whose rheumatic attacks were not preceded by such infections. Whether our patients who gave no history of respiratory infections actually had none, we cannot be certain, since we saw them, in most instances, only after the onset of rheumatic symptoms, but it can be said that these patients did not have respiratory infections of sufficient severity to be remembered by them. Our findings are largely in accord with those of Coburn and Pauli (5) although not in agreement with their report (13) that the onset of rheumatic fever coincided with a sharp rise in antistreptolysin titer. Indeed, many of our patients showed no rise until weeks after the onset of rheumatic fever and in a fair number the titer did not rise at all during periods of observation as long as 6 months. In this connection, however, it must be emphasized that some patients with such known hemolytic streptococcal diseases as scarlet fever and erysipelas show no antistreptolysin increase, so that the negative find-

ings in some rheumatic patients are of little weight in arguing against the hemolytic streptococcal theory of etiology of that disease

Since it has repeatedly been shown that a rise in the antistreptolysin level occurs only after infection with hemolytic streptococci and not after infection with other micro-organisms our results, we believe are in support of the view that there is an important relationship between rheumatic fever and hemolytic streptococcal infection. Whether the latter acts in a primary or merely in a contributory capacity however, cannot be stated with such confidence.

In view of the lack of parallelism between the height of antistreptolysin titer and the severity of rheumatic fever, as mentioned previously, the test would appear to have little value in prognosis. As an aid in the differential diagnosis of rheumatic fever, the antistreptolysin test obviously must be interpreted with caution since it is in no way specific for that disease but merely indicates a present or recent infection with hemolytic streptococci. Nevertheless, the antistreptolysin titer is so constantly increased in rheumatic fever and so generally normal in other diseases involving the joints, that we have found the procedure distinctly useful as a confirmatory test, particularly in clinically atypical cases. Whether or not measurement of the antistreptolysin titer may be helpful in deciding whether the infectious process is active or inactive in rheumatic patients is not certain. Here again the test gives no specific indication of rheumatic activity but it does afford one more bit of presumptive evidence to be interpreted in the light of the clinical and other laboratory findings.

SUMMARY

1 The results of 1539 determinations of the antistreptolysin titers of the sera of 817 patients are here reported

2 In normal individuals we have found the median titer to be 25 units and the normal maximum titer to be 100 units per cc

3 Eighty per cent of patients with scarlet fever showed a rise in antistreptolysin titer during the course of the disease, but in only 48 per cent did it rise above 100 units in the 3 to 4-week period of observation.

4 The majority of all patients with active rheumatic fever showed a high antistreptolysin titer

The incidence of elevated titers bore no relation to the various clinical manifestations of the rheumatic infection. Those patients whose illness was preceded by an upper respiratory infection sufficiently severe to be remembered, showed this immunological response more commonly than those without a history of preceding upper respiratory infection. Patients with inactive rheumatic heart disease usually had normal titers.

5 The majority of patients with chorea showed an increased titer regardless of whether or not the condition was associated with heart disease or polyarthritis. Age, severity of illness and fever therapy did not seem to influence the antibody titer.

6 Patients with rheumatoid arthritis, as a rule, did not show an abnormal antistreptolysin titer. This is in sharp contrast to the high incidence of raised hemolytic streptococcus agglutinin titers observed in these patients even when the identical strain was used as an antigen to measure both antibodies.

7 Patients with other types of arthritis similarly failed to show an elevated antistreptolysin titer in a significant percentage of cases.

8 Because three-quarters of the patients with rheumatic polyarthritis showed an abnormally high titer and those with other forms of joint diseases did not, the test was found helpful in diagnosis.

9 Patients with erythema nodosum of hemolytic streptococcal origin and patients with acute pericarditis of rheumatic type had as a rule an abnormal titer. When, however, these conditions were of a tuberculous origin, the titer was within normal limits. Here again the serological determination may be of diagnostic value. Further studies are needed to corroborate this impression since the number of cases here reported is small.

10 Several patients with lupus erythematosus disseminatus and periarteritis nodosa had a normal antistreptolysin titer.

11 In no disease have we found a correlation between the antistreptolysin titer and the severity of the illness, hence the titer is of doubtful prognostic significance.

12 Finally, it must be borne in mind that an elevated antistreptolysin titer may be found in a patient suffering with a disease not related to, but merely preceded by a hemolytic streptococcal infection.

fection To reason that the elevated titer indicates that the presenting disease is of hemolytic streptococcal origin may lead to an erroneous diagnosis

We wish to express our thanks to Mrs Rose C. Alexander for her help in carrying out the various technical procedures

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THE RELATION OF METHEMOGLOBIN TO THE CYANOSIS OBSERVED AFTER SULFANILAMIDE ADMINISTRATION

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We have stated in a preliminary report that the cyanosis observed in individuals treated with sulfanilamide is due to methemoglobin, and rarely to sulfhemoglobin (1). We recognized at the time that attempts had been made to explain the presence of cyanosis on a different basis. Marshall and Walz (2) maintained that a black pigment derived from sulfanilamide and present in the blood, was the cause of this cyanosis. A similar explanation was advanced by Ottenberg and Fox (3). They described the phenomenon of a bluish purple pigment resulting when a colorless sulfanilamide solution was exposed to ultra violet light. We have confirmed this photochemical change of sulfanilamide. The results of our investigation are in agreement with those of Hartmann and his associates who showed that the cyanosis was related to methemoglobin formation and that methylene blue administered to cyanotic patients caused a disappearance of the cyanosis and a reduction in the concentration of methemoglobin (4).

At this time we desire to present further evidence that the cyanosis is due solely to the presence of methemoglobin and in rare instances to sulfhemoglobin. We have also included the results of a study of individuals receiving sulfapyridine. Spectral distribution curves were obtained with the spectrophotometer, using normal human blood and the bloods of patients to whom sulfanilamide or sulfapyridine was being administered. In initiating such a study we believed that if any pigment other than methemoglobin that might cause cyanosis were present, it would be detected in the spectral distribution curves

because of the presence of an infection. The free sulfanilamide in their bloods was determined on the day the bloods were examined for methemoglobin. These levels ranged from 4.9 to 20.8 mgm. of sulfanilamide per 100 cc. of blood. Sulfapyridine was given in divided doses for a total of 4 to 9 grams per day. The blood levels of free sulfapyridine varied from 2 to 14 mgm. per 100 cc. of blood. Data for the absorption curves were obtained with a Martens type polarization photometer in combination with a Bausch and Lomb spectrometer. The spectrophotometric examinations were made in the range from 480 to 700 m μ . Whole blood was diluted with distilled water to give a concentration of 1/25 or 1/50. This solution was centrifuged for 10 minutes at 2400 revolutions per minute (radius 10 inches) in order to remove the stroma of the cells and clarify the solution. Absorption cells 0.5 or 1.0 cm. in length were used over the spectral range from 480 to 600 m μ and 3 cm. cells were used from 600 to 700 m μ . The slit of the spectrometer was opened fairly wide during these observations so that more light could be gathered. This reduced the height of the oxyhemoglobin absorption peak at 576 m μ a few per cent below the values which would have been obtained with a narrow slit, due to the decreased resolving power. A constant slit width was used throughout.

The first step was to obtain the normal absorption spectra for oxyhemoglobin and methemoglobin. Bloods from normal individuals were used, and dilutions were made as previously mentioned with distilled water. Oxyhemoglobin and methemoglobin spectral distribution curves were made from absorption spectra of the same samples of blood. Absorption of oxyhemoglobin at 576 m μ was arbitrarily made unity in order to cancel out the effect of concentration.

Methemoglobin was formed by the addition of an excess of potassium ferricyanide. (The amount was not measured, but approximated 100 mgm. per gram of hemoglobin.) In each instance it was readily established spectroscopically that the conversion to methemoglobin was complete.

RESULTS

Normal absorption curves for oxyhemoglobin and for methemoglobin are shown in Figure 1. These curves are the average of several that were obtained by noting the absorption of blood solutions from 3 normal individuals. From the

MATERIAL AND METHODS

Spectroscopic studies have been carried out on the bloods of 3 normal adults, 15 patients receiving sulfanilamide, and 5 more to whom sulfapyridine was given. From 4 to 8 grams of sulfanilamide were administered to the patients daily in divided doses for several days

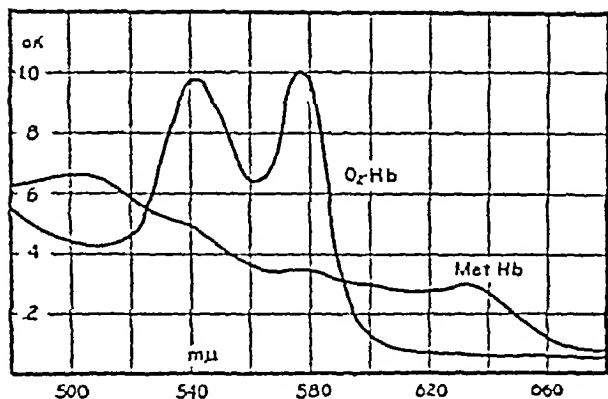


FIG. 1 ABSORPTION CURVES OF METHEMOGLOBIN AND OXYHEMOGLOBIN

Blood diluted with distilled water and centrifuged.

curves in Figure 1, it was found possible to construct curves representing bloods with given ratios of oxyhemoglobin and methemoglobin. These could then be used as a basis for a quantitative chemical analysis of blood containing methemoglobin, provided there were no other foreign colored substances present in the blood. In Figures 2 and 3 such a family of curves is shown for blood diluted with distilled water. In these figures, the values indicating the amount of absorption are made unity at 576 $m\mu$ (Figure 2) and 630 $m\mu$ (Figure 3) respectively. In other words, one could consider the values plotted to be the ratio of the absorption at any particular wavelength to the absorption of 576 $m\mu$ for Figure 2, and correspondingly at 630 $m\mu$ for

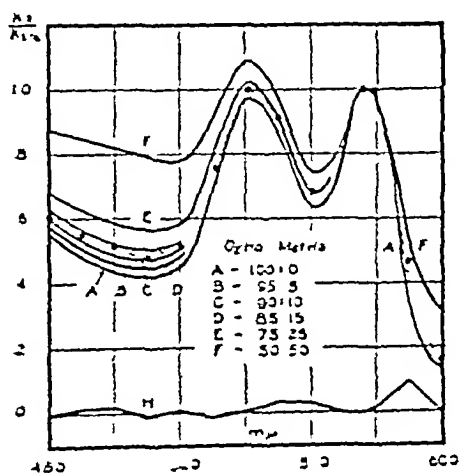


FIG. 2 RATIO OF THE ABSORPTION AT ANY GIVEN WAVELENGTH TO THE ABSORPTION AT 576 $m\mu$ FOR BLOOD CONTAINING DIFFERENT PROPORTIONS OF OXYHEMOGLOBIN AND METHEMOGLOBIN

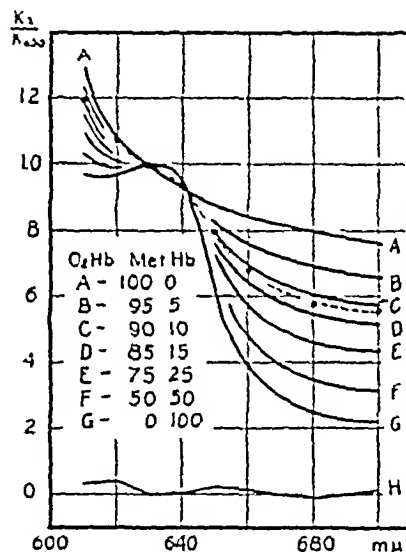


FIG. 3 RATIO OF THE ABSORPTION AT ANY GIVEN WAVELENGTH TO THE ABSORPTION AT 630 $m\mu$ FOR BLOOD CONTAINING DIFFERENT PROPORTIONS OF OXYHEMOGLOBIN AND METHEMOGLOBIN

Figure 3. In this manner, a correction is made for different concentrations of hemoglobin in various samples of blood. The characteristic absorption band of methemoglobin has a maximum at 630 $m\mu$ in neutral or slightly acid solution, which makes its identification possible if other foreign colored substances were present in the blood. Therefore, absorption curves of blood containing methemoglobin should match one of the calculated curves of Figures 2 and 3 throughout the entire spectrum. Any other colored substance could then be detected by the divergence of these curves.

Blood was obtained from a patient who was moderately cyanotic due to sulfanilamide. Spectroscopic examination of the blood revealed the presence of an absorption band at 630 $m\mu$, which is characteristic for methemoglobin. A spectrophotometric study of the same sample of blood was made, and the dotted curve seen in Figures 2 and 3 represents the absorption curve of this blood. From the position of this curve, it was estimated that this blood contained 12 per cent methemoglobin.

If the dotted curve in Figures 2 and 3 is subtracted from a theoretical curve that represents 12 per cent methemoglobin (not shown in figures), the curve of the difference is represented by H. The absorption represented by H is no

more than would be expected from experimental variations. Therefore our observations are consistent with the interpretation that no colored substance is present possessing a significant amount of absorption over the wavelengths covered other than methemoglobin and oxyhemoglobin. The blood studied in this and other experiments reported in the paper was diluted with distilled water similar results have been obtained with blood diluted in 0.4 per cent ammonia, although the absorption spectra were quite different. With a spectrophotometric method previously described, the value for the methemoglobin concentration for the sample of blood represented by the dotted curve was 10 per cent. In 7 additional instances compared close correlation was found between the results of the spectrophotometric and spectrophotometric determinations. These data were tabulated in our previous report (1). Since the spectrophotometric method depends entirely upon the characteristic absorption band of methemoglobin at $630 m\mu$ it is unlikely that such a correlation would be found to exist if pigments other than methemoglobin and oxyhemoglobin were present in appreciable quantities. The possibility might be considered that another pigment having absorption at $630 m\mu$ was present, but it would be an unlikely coincidence that, in addition to possessing very similar absorption spectra such absorption would also be completely removed by methylene blue. This is discussed later.

The amount of methemoglobin in a series of 13 determinations as calculated in a manner similar to that described by Heilmeyer (5), is given in Table I. The determinations are made from the ratio of the extinction coefficients K_{100}/K_{410} and K_{110}/K_{111} . The subscripts refer to the wavelength at which the extinction coefficient was determined.

Differences in the amount of methemoglobin as determined in the different parts of the spectrum can be ascribed to an unknown pigment or to experimental errors. The majority of the determinations of this table have had their spectral distribution curves plotted in the manner illustrated in Figures 2 and 3. In these cases the curves followed those of a calculated methemoglobin concentration quite closely throughout. Thus it is believed that pigments of unknown nature

TABLE I
Per cent methemoglobin

Number	1	2	3	4	5	6	7	8	9	10	11	12	13
K_{100}/K_{410}	7.0	8.0	5.0	10.8	11.5	16.0	12.7	18.0	11.0	9.8	1.0	10.0	0.5
K_{110}/K_{111}	12.0	7.8	8.0	9.2	11.0	21.0	14.0	25.0	18.5	15.0	0.8	10.0	1.5

are not present in sufficient quantity to contribute to the production of cyanosis. Determinations 11 and 13 in Table I were made on the bloods of patients who were not cyanotic.

A cyanotic patient, whose blood contained 12.3 per cent methemoglobin as determined from the absorption curves' calculation, was given intravenously 20 cc. of a 1 per cent solution of methylene blue. One half hour after this injection the blood of the patient was examined with the spectrophotometer and the absorption spectrum obtained. This spectrum was that of normal blood (determination 11 of Table I). So it would appear that the methylene blue caused the removal of methemoglobin as well as other colored substances, if the latter were present. It is important to point out that the introduction of methylene blue into this patient's blood caused a decrease of about 20 per cent of the absorption due to oxyhemoglobin content during this half hour period. This may well account for the temporary hyperpnea these patients exhibit shortly after the injection of methylene blue, usually lasting for 15 to 30 minutes.

The blood of another patient who was being treated with sulfanilamide was studied. He had no evidence of cyanosis at the time that his blood contained 13 mgm per 100 cc. of free sulfanilamide. An absorption spectrum of his blood was obtained at this time showing an approximately normal spectrum. The methemoglobin concentration was about 1 per cent (determination 13 Table I).

It is of interest that over 50 patients have been observed while being treated with sulfapyridine. In but 1 of these patients has cyanosis been noted that could be attributed to the drug. This patient had a Type XXVI pneumococcus pneumonia involving the right lower lobe with an associated massive collapse of the same lobe. Sulfapyridine was administered and after 48 hours mild cyanosis was noted. At this time, spectroscopic examination of laked blood (15

water) revealed a very weak absorption band at $630\text{ m}\mu$ (maximum). When the same sample of blood was examined spectrophotometrically, the peak of the absorption curve was in the same range as for methemoglobin. One difference in the curve was noted. Absorption was slightly increased in the whole region of $600\text{ m}\mu$ to $700\text{ m}\mu$, as compared to the bloods from patients receiving sulfanilamide. With the spectrophotometric method described above, the value for methemoglobin (assuming that the total absorption was due only to met- and oxyhemoglobins) was 7 per cent. Thirty minutes after the patient had received 18 cc of 1 per cent methylene blue solution intravenously, examination of the blood showed the normal absorption spectrum for oxyhemoglobin. The presence of methemoglobin in the blood of this individual, who received sulfapyridine, appears to be in agreement with the more extensive observations of Barnett and his associates (9).

The absorption spectra of the bloods of 3 other individuals receiving sulfapyridine were studied and were found to be quite close to normal, even when the blood contained as much as 5.4 mgm of free sulfapyridine and 1 mgm of the acetylated form per 100 cc. There was no methemoglobin absorption. In these spectral distribution curves there was a little greater absorption of quite uniform magnitude between 600 and $700\text{ m}\mu$ and for a given amount of hemoglobin, as measured by the $576\text{ m}\mu$ absorption peak, there was a slightly greater than normal absorption in the region around $500\text{ m}\mu$. This may indicate the presence of a small amount of an unknown pigment.

The absorption spectrum was determined for the bluish-purple pigment that results when dilute solutions of sulfanilamide are irradiated with ultra violet. This spectrum exhibits a broad absorption with a maximum at $570\text{ m}\mu$, from which point it decreases gradually throughout the visible spectrum. Its presence, when mixed with blood, would be detected by an increase in absorption in the red region of the spectrum. There should be no difficulty in separating methemoglobin from this pigment.

DISCUSSION

As noted in our preliminary report (1), the blood from patients who have become cyanotic

after receiving sulfanilamide has always been found to contain methemoglobin (or rarely sulfhemoglobin) when examined in sufficient concentration. We believe that failure by others to observe methemoglobin in such instances is probably on the basis of too great dilution of the laked blood. Thus we have noticed on a number of occasions that the methemoglobin band could not be detected in blood diluted 1:20 or even 1:10 with distilled water, but that it was distinctly visible in a dilution of 1:5. (This applies to a 2 cm thickness with a Zeiss grating spectrometer.) The present spectrophotometric study has failed to reveal the presence of any pigment other than methemoglobin in the blood of cyanotic patients receiving sulfanilamide. On the other hand, neither cyanosis nor methemoglobinemia has been noted following sulfapyridine therapy. In this connection, it is of interest that we have not been able to affect sulfapyridine with ultra violet light. Pigments such as those derived from sulfanilamide under similar conditions do not appear. The significance of this difference is not clear, but it may be that methemoglobin formation is dependent upon derivatives of sulfanilamide which are not formed in the body from sulfapyridine.

Webb and Kniazuk (6) have recently reported the presence of pigments other than met- or sulfhemoglobin (although these were also noted at times) in the blood of rats receiving sulfanilamide. In comparing their results with those of the present study, several points deserve mention. The difference in species may or may not be of significance. The amount of sulfanilamide which they used was 15 to 40 times that used in the patients of the present series. It would be interesting to know whether the concentration of sulfanilamide in the rats' blood was in any way correlated with Webb and Kniazuk's finding of pigments other than met- or sulfhemoglobin. They did not report data on the blood sulfanilamide level. The very large doses which they used evidently were often productive of severe toxic effects since they note that "when there seemed to be danger of the animal dying, the dose was reduced to 1.5 grams per kilo" (from 2 grams per kilo). Severe toxicity, on the basis of such massive dosage, is a factor of unknown significance with respect to pigment metabolism, and this factor was absent in the present study.

Wendel and Wendel (7) have recently reported their results of spectrophotometric and gasometric measurements of the bloods from cyanotic patients receiving sulfanilamide. Their data indicate that methemoglobin is the principal if not the sole source of the abnormal color of the blood. On the other hand, Bigler and Werner (8) using a gasometric method found that appreciable cyanosis may occur without the presence of inactive hemoglobin. Likewise cyanosis occurred without methemoglobin or sulfhemoglobin being demonstrated. It is difficult to interpret the results of Bigler and Werner, since they do not state the dilution of blood examined spectroscopically or the thickness of the tube used.

SUMMARY AND CONCLUSIONS

1 The cyanosis observed in man following sulfanilamide therapy is explained by the presence of methemoglobin (rarely sulfhemoglobin)

2 Methylene blue abolishes the cyanosis due to methemoglobin (and other pigments if present, except sulfhemoglobin), and the spectral distribution curve of the blood becomes normal

3 Spectrophotometric studies of the blood of sulfanilamide treated patients have failed to reveal the presence of pigments other than methemo-

globin in quantities large enough to contribute appreciably to cyanosis.

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THE RENAL EXCRETION OF INULIN AT LOW PLASMA CONCENTRATIONS OF THIS COMPOUND, AND ITS RELATIONSHIP TO THE GLOMERULAR FILTRATION RATE IN NORMAL, NEPHRITIC AND HYPERTENSIVE INDIVIDUALS¹

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Precise measurements of many of the physiological characteristics of the kidney depend on the principle that the clearance of any freely filtrable compound which is excreted only by the glomeruli is identical with the filtration rate. Rehberg, the first investigator to apply this principle to the human kidney suggested the use of exogenous creatinine as a measure of the filtration rate (1). Later it was demonstrated that *exogenous* creatinine is excreted by the tubules as well as the glomeruli of the human kidney (2). Many other compounds have been suggested for the measurement of glomerular filtration rate in man but of these only inulin has achieved wide acceptance. The evidence supporting the inulin clearance as a measure of filtration has been reviewed extensively by Smith (3), and needs no detailed repetition. The evidence so far presented is not entirely conclusive and consists chiefly, if one omits the rather questionable evidence based on consideration of the diffusion characteristics of inulin and its behavior in species far removed from the human of three types of observations: (a) when tubular function is depressed by phlorizin, or when the tubular cells are "saturated" with glucose, ascorbic acid etc., the clearances of a number of compounds approach the value of the inulin clearance (3); (b) the inulin clearance remains constant when the plasma concentration of inulin is varied over a wide range (4), and (c) in a certain number of cases the specifically determined, endogenous creatinine clearance is equal to the inulin clearance (5).

The evidence supporting the inulin clearance would be much strengthened if several compounds of different molecular configuration could be shown to possess the same excretion characteristics as inulin. Such correspondence has been

demonstrated in the dog for inulin, creatinine and ferrocyanide, but the same relationship does not hold for the human kidney.²

Shannon and Smith (4) have rightfully stressed the importance of the fact that the inulin clearance in normal and phlorizinized man is independent of the plasma concentration. In their experiments they varied the plasma concentration from 50 to 400 mgm per 100 cc. and observed complete independence of the clearance values in this range. However we feel that these excellent observations need amplification in two respects: one, the observations do not include patients suffering from nephritis or hypertension, and the other, that in the range of relatively high plasma concentration which they studied, these authors could have observed the effects of only rather considerable amounts of tubular excretion or reabsorption. That is, upon consideration of minimal variations in clearances from period to period and also of the minimal errors involved in the numerous analytical manipulations it appears that secretion or reabsorption of 5 mgm. per minute of inulin could not have been noted by these authors and possibly quantities somewhat larger might have escaped detection. It can be shown by a simple calculation, as developed later in the section on discussion, that such quantities of secreted or reabsorbed inulin could be detected readily by clearance studies at plasma levels below 20 mgm. per cent. Previous analytical methods for the determination of inulin were inadequate for study of inulin clearances at such low plasma concentrations. Recently a sufficiently sensitive and precise method has been introduced by Alving, Rubin and Miller (6). With this method we have been able, in the pres-

¹ Aided by a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

² Recent preliminary studies by Smith (10) suggest that sorbitol and mannitol are excreted "in" entirely by glomerular filtration.

ent paper, to make the needed comparison of the inulin clearances at high and very low plasma levels in normal, nephritic and hypertensive individuals

Calculation of the clearances

Inulin clearances were obtained by dividing the urinary excretion of inulin per minute by the concentration in the serum at the mid-point of the period. In those experiments in which constant infusions were given the blood was drawn at the mid-point of the period. In others, in which a single dose of inulin was injected, a number of blood samples were analyzed and their concentrations plotted on semi-logarithm paper. The mid-point concentrations were obtained from the graph and it was sufficiently accurate for our purposes to take this mid-point concentration as the average for the period.

Urea clearances were calculated by the usual formula of UV/B whenever the urine volume was greater than 2 cc. per minute, as it was in almost all the periods of every experiment but one. In the one experiment where the urine volumes were below the "augmentation limit" the urea clearances were calculated as $U\sqrt{V}/B$

Analytical methods

Fresh urine specimens and blood serum were employed for all the analyses.

Inulin Analyses of inulin were performed on cadmium filtrates by the colorimetric method of Alving, Rubin, and Miller, with the single modification of the substitution of a Number 635 Evelyn filter in place of the 660 filter recommended originally. The acid-alcohol, diphenylamine reagent was made fresh each day. Glucose in the serum and urine was removed by fermentation with yeast, fermentation is necessary when the urine inulin concentration is low, even in the presence of a negative Benedict's test for glucose.

To obtain as precise inulin clearance values as possible, the urines were diluted to contain nearly the same concentration of inulin as the corresponding serum filtrates.

Urea The urea in the serum filtrates was determined by the hypobromite method of Van Slyke and Kugel (7) and the urea in urine by the urease method of Van Slyke (8)

Plan of experiments

Two types of experiments were performed. In one, continuous infusions of inulin were given to maintain constant plasma levels, and in the other type a single injection of inulin was made and the clearances obtained on a falling curve of plasma concentration. For both types of experiments certain technical details were the same, as follows. The subjects were kept in bed for 12 hours before the experiment. Breakfast consisted of toast, butter, and one-half glass of milk. 200 cc. of water were given every half or 1 hour for 3 hours before the first clearance period and also during the experiment.

Urine specimens were collected by catheterization, followed by three washings of the bladder with 20 cc. portions of physiological saline. The washings were added to the urine obtained by catheterization. Blood samples were obtained by venipuncture. In almost every experiment an active diuresis was obtained, and except for one experiment the urine volumes were considerably above 2 cc. per minute.

In the experiments with constant inulin plasma levels, a priming dose of inulin was given and this was followed immediately by the continuous injection of a dilute solution of inulin in physiological saline. The quantities were calculated to give in the first plateau a constant level of approximately 5 mgm. per 100 cc. of plasma. The sustaining solutions were given at the rate of 2 cc. per minute by means of a special pump designed by Dr. H. R. Jacobs. Thirty minutes were allowed for equilibrium, then two or three clearance periods of 20 to 30 minutes' duration were obtained. Another dose was then given to raise the plasma concentration to the desired level, and the sustaining solution replaced with a more concentrated one. Again after the equilibrium period several more clearance periods were obtained.

In the other type of experiment, 10 grams of inulin in 100 cc. of physiological saline were administered intravenously. Blood samples were drawn at 45 minutes, 1 hour, 15 minutes, 1 hour, 45 minutes, 2 hours, 15 minutes, and 3 hours after the initial injection. Urine collections were begun 30 minutes after completion of the inulin injection, and were so timed that the above blood samples fell at the mid-points of the clearance periods.

RESULTS

Table I presents a summary of experiments on 6 normal individuals. In the first 3 experiments the inulin clearances were obtained at plasma levels maintained by constant intravenous injection, and in the other 3, after a single intravenous injection of inulin. It is apparent that the inulin clearance at very low plasma levels corresponds closely to the values obtained at relatively high concentrations. In the first 3 experiments the average clearance at the lower plasma level is 12 per cent to 17 per cent lower than the average at the higher concentration. These differences are small when contrasted with the 900 to 1400 per cent increase of plasma concentration in these experiments. It is significant that in each of the first 3 experiments, at least one period in each lower plasma plateau had as low a urea/inulin clearance ratio as observed at the higher plasma level. This indicates that there is no real tendency for the inulin clearance to drop to a lower value with the decrease in plasma concentration.

TABLE I

Clearances at high and low plasma concentrations of inulin in normal individuals

Subject	Inulin		Ratio urea/inulin clearance
	Plasma concentration	Clearance	
	mgm. per 100 cc.	cc. per min. per 1.73 sq m.	
D K. Male 23 years	4.53	101	0.60
	4.55	120	0.55
	56.1	120	0.56
	51.9	116	0.54
	49.6	140	0.53
M B Male 21 years	10.9	105	0.65
	9.89	123	0.54
	96.4	131	0.48
	94.5	125	0.52
B E Male 22 years	3.3	121	0.50
	3.6	121	0.43
	50.9	150	0.50
	45.7	134	0.48
A. W Male 31 years	6.6	113	
	10.5	111	
	14.3	119	
	21.5	128	
	35.0	130	
L B Female 36 years	7.07	105	
	10.6	109	
	14.3	103	
	20.8	89	
	34.7	91	
L A Male 44 years	10.3	93	
	14.6	76	
	18.3	91	
	25.4	74	
	37.3	86	

If such a tendency did exist one would anticipate a consistent rise in the urea/inulin clearance ratio

In the other 3 experiments performed after injection of a single dose of inulin the results demonstrate the same tendency for the inulin clearance to remain at low plasma levels the same absolute value as at the higher concentrations. In 2 of the 3 experiments (L. B and L. A) the clearances are slightly higher at the low levels than at the higher plasma concentrations. This was due most likely to changes in the filtration rate during these periods rather than to a specific effect of the low plasma concentration on the clearances. Since urea clearances were not obtained, these experiments are somewhat less satisfactory than the previous ones in which the urea/inulin ratio gives

a relatively satisfactory index of any changes in the filtration rate that might have occurred from period to period. However, these 3 experiments demonstrate clearly that the inulin clearance at plasma levels of 5 to 10 mgm. per 100 cc. shows no consistent deviation from the clearances obtained at levels four to five times higher

In Table II a summary is presented of the results obtained on 6 patients suffering from various types of nephritis. The results are essentially the same as those obtained with the normal indi-

TABLE II

Clearances at high and low plasma concentration of inulin in individuals with various types of nephritis

Subject	Diagnosis	Inulin		Ratio urea/inulin clearance
		Plasma concentration	Clearance	
		mgm. per 100 cc.	cc. per min. per 1.73 sq m.	
V S Female 23 years	Nephrosis	4.81	86.1	0.63
		4.70	86.8	0.58
		70.9	110	0.59
		64.8	97.2	0.45
E S Male 25 years	Chronic glomerulonephritis	4.1	60.0	0.77
		4.4	55.0	0.76
		10.0	64.0	0.57
		11.4	59.0	0.61
		71.8	68.0	0.58
A T Male 21 years	Chronic glomerulonephritis	8.03	22.1	0.41
		7.83	16.5	0.47
		6.98	14.1	0.46
		85.2	13.5	0.33
		83.6	16.3	0.35
M C Female 37 years	Chronic glomerulonephritis	82.5	9.3	0.37
		7.7	22.1	0.80
		7.4	20.8	0.88
		7.4	19.0	0.82
		67.1	23.5	0.79
M H Female 39 years	Nephrosis	61.5	20.3	0.79
		61.2	22.2	0.77
		6.9	93.3	
		10.0	107.4	
		13.0	122.6	
B E. Male 20 years	Subacute glomerulonephritis	19.1	85.8	
		27.7	110.8	
		8.7	92.8	
		13.3	88.5	
		18.4	89.3	
		25.5	103.0	
		36.0	80.7	

individuals and show the independence of the inulin clearance from plasma concentration. The cases studied show considerable variety in the clinical picture of Bright's disease. Two cases (A T and M C) were typical advanced cases of chronic glomerulonephritis with marked loss of renal function. One patient (B E) had the disease for 4 months without much impairment of renal function, the remaining patient with glomerulonephritis (E S) had the disease for 4 years, and at the time of the experiment showed about 50 per cent of normal kidney function. In the other 2 patients (V S and M H) the diagnostic criteria corresponded to those of so-called genuine lipoid nephrosis, however, as generally turns out to be the case, these patients will probably be shown eventually to have been in the nephrotic stage of chronic glomerulonephritis.

The results of experiments on 3 patients suffering from severe essential hypertension are given in Table III. The inulin clearances in 2 of the patients (M F and B H) have the same values at the low plasma level as at the higher ones. In the remaining patient (L M) the absolute values of the clearances obtained at 4.2 to 4.5 mgm per cent are lower than those at 38 to 50 mgm per

cent. These differences appear to be due to a specific effect of the plasma level of inulin since there was an elevation of the urea/inulin ratio at the lower level. In the third period of the low plasma plateau the effect is very marked, and the inulin clearance in this period is about 50 cc below the next period obtained at a much higher plasma concentration. Even so, as brought out later in the discussion, this unusually low clearance, which represents an exceptional case in our experiments, would indicate the reabsorption of only 2 mgm of inulin per minute.

DISCUSSION

The experiments described in this paper were designed to detect possible tubular excretion or reabsorption of inulin by the kidney of normal human subjects and also in individuals suffering from nephritis and hypertension. It is axiomatic that the amount of an inert compound excreted entirely by glomerular filtration has within certain wide limits no maximum, and its excretion is directly proportional to plasma concentration. However, since tubular excretion and reabsorption are in most cases dependent on specific activities of the tubule cells, these processes tend to have well-defined maximal rates. Thus filtration may easily mask the effect of tubular activity if proportionality between clearance and plasma concentration is studied at high plasma values. This phenomenon, and the rationale of our experiments, can be illustrated best by the calculations shown in Table IV. As pointed out in the introduction, it is reasonable to assume that the tubular excretion or reabsorption of 5 mgm per minute of inulin could not have been detected by

TABLE III

Clearances at high and low plasma concentration of inulin in individuals with essential hypertension

Subject	Inulin		Ratio urea/inulin clearance
	Plasma concentration mgm per 100 cc	Clearance cc. per min per 1.73 sq m	
M F Male 46 years	61	71.4	0.59
	5.6	76.4	0.52
	65.9	69.0	0.54
B H Male 25 years	5.7	122.0	
	5.7	103	
	13.9	102	
	12.4	107	
	36.9	103	
L M Female 33 years	32.7	118	
	4.5	95.2	0.64
	4.5	94.4	0.58
	4.2	63.8	0.50
	39.7	117.8	0.47
	43.9	113.6	0.53
	33.0	99.1	0.45

TABLE IV

Deviations of the inulin clearance from the true filtration rate that would result from tubular excretion or reabsorption of a small quantity of inulin (a filtration rate of 100 cc per minute is arbitrary, chosen)

Plasma inulin mgm per 100 cc	Inulin clearance	
	Assuming tubular excretion of 5 mgm inulin per minute	Assuming tubular reabsorption of 5 mgm inulin per minute
100	105	95
20	125	75
5	260	0

previous experiments which were conducted at relatively high plasma concentration. If for example one assumes a true filtration rate of 100 cc. per minute at a plasma value of 100 mgm per cent (a value used very frequently in experiments with inulin) then the assumed amount of tubular excretion or reabsorption would have caused the clearance to change to 105 cc. or 95 cc. respectively. It is doubtful whether the analytical methods employed would have detected the change, or if they had whether any particular significance could be attached to a 5 cc. per minute variation in clearance from period to period.

However if the clearances had been determined at 20 mgm per cent plasma concentration, then the 5 mgm tubular excretion or reabsorption of inulin would have caused the clearance to change to 125 cc. and 75 cc. respectively. These deviations from the true filtration level of 100 cc. could be detected. At a plasma level of 5 mgm. per cent the 5 mgm tubular excretion or reabsorption would give clearances of 200 or 0 respectively!

The results of our experiments show that at plasma levels in the range of 5 mgm. per cent the inulin clearance has essentially the same value as at the high ranges of plasma concentration. This appears to rule out definitely the possibility of any appreciable amount of active tubular excretion or reabsorption of a fixed quantity of inulin. In a few experiments, it is true, the clearance values were slightly lower at the low plateau than at the high one. For the normal individuals the experiment on B. E. shows the greatest difference between the clearance at low and high plasma values. Assuming that the difference between the clearances of 121 and 150 was caused by tubular reabsorption of inulin then the amount reabsorbed would be $29 \times 0.036 = 1$ mgm. per minute. It is problematical whether even such a slight quantity of reabsorption occurred in this experiment because the urea/inulin ratios were relatively constant throughout the entire experiment. Thus it appears from the experiments on normal individuals that there is no tubular excretion of inulin and very little, if any, reabsorption of inulin by the normal kidney. Similarly it can be said from the experiments on 6 nephritic individuals that there is no greater tendency to tubu-

lar excretion or reabsorption in the nephritic than in the normal kidney.

In 2 of the individuals with essential hypertension there was no evidence of tubular excretion or reabsorption of inulin. In the third case (L. M.) the last clearance obtained at the low plasma concentration was exceptionally low, 49 cc. below the first clearance at the high plasma plateau. This low clearance could have been caused by the reabsorption of $49 \times 0.042 = 2.1$ mgm of inulin per minute. The analyses for this period were rechecked and found to be correct. Also the urea/inulin clearance ratio rose suddenly in this period to the unusually high value of 0.80. The high ratio could conceivably be caused by the reabsorption of an unusually small percentage of the filtered urea but this seems improbable because the urea clearance agreed with the values for preceding and succeeding periods. Hence, this period probably represents an exceptional example in which the kidney tubules reabsorbed a slight, but definite, quantity of inulin. It is probably fortuitous that this exceptional result was found in a patient with hypertension rather than in a normal or nephritic individual.

It is possible to raise an obvious objection to the type of reasoning employed in these experiments. We have assumed that if tubular excretion or reabsorption of inulin does occur, the maximum amount of inulin capable of crossing the tubules would be a relatively fixed quantity per minute. One may argue that the tubular reabsorption or excretion of inulin would be proportional to the concentration in the plasma—that is, a constant percentage of the inulin brought to the kidneys would be transported across the tubule cells. Such an hypothesis cannot be excluded though no data support it. However the extensive evidence now available on the function of the tubules indicates that they can excrete or reabsorb relatively fixed maximal quantities of a number of compounds (9). Thus the basic assumption which is made in this paper regarding the excretion of inulin is entirely compatible with the known facts.

It may be asked whether it is worthwhile to ascertain if the inulin clearance is a precise measure of the filtration rate. Would not the clearance be as satisfactory for physiological and clinical measurements if it were within 5 per cent of

the true value? In most cases the absolutely precise value may not be necessary, but it becomes highly important whenever one investigates tubular activity at high plasma concentrations of a given compound. For example, in ascertaining the maximum quantity of exogenous creatinine excreted by the tubule cells (2), it may be necessary to subtract a filtration value of 120 from a creatinine clearance of 135 to obtain the "tubular clearance." It is apparent that even a few per cent uncertainty in the true filtration rate would introduce large percentage errors in the estimation of the tubular clearance.

The demonstration that the inulin clearance at low plasma concentration has the same value as at the high levels previously employed leads to certain practical applications. In the first place it permits one to do experiments after the injection of relatively small quantities of inulin. This reduces the dangers from reaction to the pyrogenic material which is found sometimes in the most carefully purified inulin. Also, reduction of the quantity of inulin represents a considerable saving of money when many experiments are performed. Another important advantage is the opportunity afforded to follow the inulin clearance for very long periods of time after a single intravenous injection of inulin. And finally, one may determine glomerular filtration rate very simply in the normal or diseased human kidney. The details of a simplified clinical procedure for the measurement of glomerular filtration rate will be described elsewhere.

SUMMARY

Inulin clearances have been determined at very low plasma levels by the sensitive, colorimetric method devised by Alving, Rubin and Miller for this purpose. As shown by calculation, small quantities of tubular excretion or reabsorption of inulin would cause the inulin clearance at low plasma levels to deviate markedly from the values obtained at high plasma concentrations. Our results demonstrate that the inulin clearance in the range of 5 mgm per 100 cc of plasma closely approximates the clearance obtained at much

higher plasma concentrations. The results indicate that in most cases there is no tubular excretion or reabsorption of inulin. In an occasional clearance period there was a possible reabsorption of 1 or 2 mgm of inulin per minute, amounts which may be considered negligible for most purposes.

Studies have been made on normal individuals and patients with severe essential hypertension and various types of nephritis. Our results support the evidence, accumulated chiefly by H. W. Smith, J. A. Shannon, and their collaborators, for the inulin clearance as a precise measure of the glomerular filtration rate in the normal human kidney, and strengthen the evidence for the diseased kidney.

The authors are indebted to the Pfanstiehl Company for generous gifts of inulin.

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THE CONTROL OF THE DOSAGE OF ANTISERUM IN THE TREATMENT OF PNEUMOCOCCAL PNEUMONIA I A STUDY OF THE MECHANISM OF THE SKIN REACTION TO TYPE SPECIFIC POLYSACCHARIDE

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In 1917 Dochez and Avery (1) isolated from cultures of actively growing pneumococci and from the blood and urine of patients suffering from pneumonia a soluble substance which possessed the property of precipitating homologous antibody. Finding that this "specific soluble substance" neutralized the protective action of antipneumococcal immune bodies, Cole (2) in the same year, advanced an important hypothesis. He suggested that antiserum administered therapeutically could not be expected to influence the course of pneumococcal pneumonia until an excess of antibody had been given over and above that needed to neutralize the specific soluble substance circulating in the patient's blood. The specific soluble substance is now known to be a complex polysaccharide derived from the capsule, specifically antigenic, and chemically unique for each type of pneumococcus. More recently it has been found in high concentration in pneumonic pulmonary exudates (3) and has been shown to nullify the opsonizing properties of homologous antibody *in vitro* (4), as well as neutralize its protective action *in vivo*.

The importance of the hypothesis advanced by Cole lies in the fact that the quantity of polysaccharide to be neutralized in the blood of patients with pneumonia varies within extremely wide limits. The production of polysaccharide depends upon many factors including the duration and extent of the infection, the rapidity with which the pneumococci are multiplying in the lung, invasion of the blood stream and the polysaccharide-producing power of the particular type of pneumococcus causing the pneumonia.¹ These com-

plex variables make it impossible to predict the "neutralizing dose" of antibody for any individual patient. Unfortunately, the logical concept of the interaction of antibody and polysaccharide has been almost entirely ignored by clinicians as evidenced by the fact that only rarely is any attempt made to control the dosage of antibody in the serum treatment of pneumonia. It is still a common practice to give antiserum according to arbitrary rules based upon specified clinical criteria (6). As will be illustrated by cases encountered during the course of the present study (7) variations in antibody requirements are too great to be defined even approximately by any such rules.

The failure of clinicians to adopt methods of controlling the dosage of antiserum may best be explained by the fact that the usual serological tests designed to detect the presence of antibody in the blood are not practical. Agglutination methods entail the use of specific antigens, usually fresh cultures or vaccines, for each type of pneumococcus and require a sample of the patient's blood serum for every determination. Precipitation tests are open to similar criticisms, and at times the significance of a positive reaction with either method is extremely difficult to evaluate, since agglutinins and precipitins may occasionally be detected in the blood before sufficient antiserum has been administered (6, 7, 8).

In 1933 Francis (8) recommended as a guide to serum therapy a skin test with the specific capsular polysaccharide of the pneumococcus. Two years earlier Tillet and Francis (9) had shown that pneumococcal polysaccharides when injected intradermally into patients convalescent from pneumonia caused an immediate wheal and erythema skin reaction appearing in 10 to 20 minutes and fading after 1 to 2 hours. The polysaccharide causing the reaction was found to

¹It has been shown by Ward (5) that the polysaccharide producing power of different types of pneumococci varies appreciably. Type III pneumococcus, for example, produces considerably more polysaccharide than type I.

be always homologous in type to the pneumococcus causing the pneumonia. The patient's capacity to react became manifest coincident with recovery from the infection and was invariably associated with the presence of type-specific antibodies (agglutinins and precipitins) in the blood. Francis and Tillett in 1931 (10) extended their observations and reported that recovery from type I pneumonia in serum-treated cases was associated with the development of a positive skin reaction to type I polysaccharide. In fatal cases, however, treated with type I antiserum, although type-specific antibodies were sometimes present in the blood, the skin test remained negative. In every case where the skin test became positive the patient recovered.

These early observations suggested that the skin test with capsular polysaccharide might serve to indicate when serum therapy could safely be discontinued. After employing the test in forty-eight cases of serum-treated type I pneumonia, Francis (8) concluded that the skin test was a valuable guide to serum therapy and a definite prognostic aid. He pointed out that the test was far simpler and more practical than agglutination tests and that it possessed the additional advantage of serving as more than a mere index of circulating antibodies, a positive reaction being apparently the resultant of the presence of specific antibody in the blood and a state of reactivity of the skin associated with recovery. "When positive," he wrote, "it invariably denotes that recovery has begun, when negative it indicates further serum therapy."

Although the important observations of Francis were published in 1933, the skin test with capsular polysaccharide has been used very little as a guide to serum treatment. Abernethy (11) and MacLeod, Hoagland and Beeson (12) at the Hospital of the Rockefeller Institute have extended and confirmed the studies of Francis, and the test has been used routinely in that hospital for several years. Elsewhere, however, it has found little favor. The apparent disrepute into which the test has fallen at the hands of other writers (6, 13, 14, 15) would seem to be explained by the fact that the mechanism of the Francis skin reaction is not clearly understood, and its several limitations have not been carefully enough defined to prevent misinterpretation.

The purpose of the present investigation has been to determine the various factors which control the skin reaction to type-specific pneumococcal polysaccharide and, by a detailed clinical study, to evaluate further its advantages and disadvantages as a practical guide to serum therapy. The results of the clinical study will be reported in a second publication (7).

METHODS

Specific capsular polysaccharides. The type-specific capsular polysaccharides of pneumococci of types I-VIII and XIV were kindly furnished by Dr. L. D. Felton of the National Institute of Health. They were prepared according to the methods of Felton (16), and none of the final products gave positive reactions for protein or contained "C" fraction of the pneumococcus when tested against heterologous serum.² The polysaccharides were dissolved in normal salt solution at a dilution of 1:10,000 and were stored in the ice box.

Skin tests with polysaccharide. Skin tests were performed by injecting 0.1 cc. of a solution of polysaccharide into the skin of the volar surface of the forearm or upper arm. (In a few cases the pectoral area and epigastrium were also used.) As a control, 0.1 cc. of normal salt solution was always injected a few centimeters from the site of injection of the polysaccharide. All injections were intradermal and the tests were read after 15 to 30 minutes. A test was considered positive only when there appeared a definite wheal surrounded by an area of erythema.

Antipneumococcal serum. Antipneumococcal serum was supplied by the Lederle Laboratories, Inc. and E. R. Squibb and Sons. Serum generously donated for "clinical trial" was used almost exclusively and included both horse and rabbit serum. The antibody titers of the sera varied from 1,000 to 10,000 units per cc. After a small initial dose, serum was injected intravenously at hourly intervals in doses varying from 10,000 to 100,000 units of antibody. A skin test was performed before each injection of serum. As soon as a positive skin reaction was obtained, regardless of the clinical status of the patient, all serum therapy was discontinued and under no circumstances was it resumed unless the skin test became negative.

Skin tests with polysaccharide and serum. A limited number of skin tests were performed in which both type I polysaccharide and various dilutions of type I antipneumococcal rabbit serum were injected into the same site. The details of these tests are described below. They were performed only on normal individuals who failed to react to the intradermal injection of type I polysaccharide alone.

² MacLeod, Hoagland and Beeson have emphasized the importance of using preparations which contain no "C" fraction of the pneumococcus if false positive reactions are to be avoided.

Passive transfer tests Passive transfer tests were done with the blood serum of patients suffering from type I pneumonia, the blood being drawn both at the time of admission to the hospital and after the polysaccharide skin test had become positive under treatment with antiserum. The donor's serum was injected intradermally into normal recipients who did not react to type I polysaccharide alone. The injection of donor's serum often caused a local erythematous reaction which faded in a few hours. The local reaction was allowed to subside before 0.1 cc. of type I polysaccharide was injected into the same site. As a control, a similar test was done with a heterologous polysaccharide (type II or type III). All reactions were read as described above for the routine skin test with polysaccharide.

Mouse protection tests Protective antibodies in the serum of patients under treatment were measured by mouse protection tests. A pure-bred strain of mice (C.F.1) was used in all experiments. The mice were injected intraperitoneally with 0.5 cc. of various dilutions of a 14 hour blood broth culture of type I pneumococci immediately after having received by the same route 0.5 cc. of a 1 to 5 dilution of the serum to be tested. The dilutions of culture varied logarithmically from 1:5 to 1:50,000,000. Five mice were injected with each dilution of culture. The type I pneumococcus used was supplied by Dr. Felton and after several passages through mice was highly virulent, 10^{-8} cc. of a 14 hour culture killing mice regularly in 20 hours. The virulence of the culture was tested in every experiment. Each test was terminated at the end of 96 hours and all mice living at the end of this time were counted as survivors. The final results were expressed in units* of antibody per cc. of patient's blood.

Agglutinin tests Tests for agglutinins were done by the Sabin microscopic method (17).

RESULTS

I The nature of the skin reaction to polysaccharide

During the early studies of the Francis skin test it was found that positive reactions to a given type of polysaccharide occurred only when agglutinins and precipitins were present in the blood (8, 9, 10). It is well known that pneumococcal polysaccharide reacts *in vitro* with antibody of the same type to form a precipitate (precipitin reaction). These two facts suggest that the Francis skin reaction may be the result of a local union of polysaccharide and antibody in the skin at the site of injection of the polysaccharide. To test this hypothesis an attempt was made to produce in normal individuals an immediate wheal

and erythema skin reaction by injecting antibody and polysaccharide into the same site. This was done in the following experiment.

Five normal subjects were selected who failed to react to the intracutaneous injection of types I, II and III polysaccharides*. Type I antipneumococcal rabbit serum containing 10,000 units of antibody per cc. was diluted with normal salt solution to concentrations of 1:10, 1:100, 1:1,000 and 1:10,000. One tenth cc. of each dilution of antiserum was injected into the volar surface of the forearm and 30 minutes later 0.1 cc. of the solution of type I polysaccharide was injected into the same sites. On the opposite arm a control experiment was carried out simultaneously with the same dilutions of type I antiserum and a heterologous polysaccharide (type II or type III). All five subjects reacted in exactly the same manner as summarized in the following table.

TABLE I
Reactions to intradermal injections of type I antipneumococcal rabbit serum and capsular polysaccharides

Dilution of serum	1:10	1:100	1:1000	1:10,000
Type I polysaccharide	+	+	+	-
Type II or type III polysaccharide	-	-	-	-

* The intradermal injection of a 1:10 dilution of rabbit serum often causes a mild local erythematous reaction but this does not interfere with the reading of the polysaccharide skin reaction if a suitable control is available for comparison.

The positive reactions observed in these tests simulated exactly the characteristic immediate wheal and erythema reaction of the Francis skin test. It should be emphasized that the reactions were produced in normal individuals who had not recently had pneumonia and who did not react to intradermal injections of the type I polysaccharide alone. A positive reaction resulted only when the antibody and polysaccharide injected were of the same type and only when the antibody was introduced in sufficient concentration (Approximately 1 unit or more in the 0.1 cc. injected.) The dilution of polysaccharide injected was not varied.

* As shown by Alston and Lowdon (18) a considerable number of normal individuals may react to pneumococcal polysaccharides.

* One unit of antibody protects a mouse against 1,000,000 lethal doses of pneumococci.

To test further the hypothesis that the Francis skin reaction results from a local union of antibody and polysaccharide in the skin, an attempt was made to transfer passively a positive reaction. The usual passive transfer technique was used as outlined above. Blood serum of four patients with type I pneumonia was obtained before and after treatment with specific antiserum. The serum drawn before treatment in each case contained no type I agglutinins, whereas that taken after treatment (when the Francis skin test had become positive) strongly agglutinated type I pneumococci. One-tenth cc of the donor serum, taken before and after treatment, was injected intracutaneously into recipients who were non-reactors to type I polysaccharide. After the local erythematous reactions caused by the serum injections had subsided, 0.1 cc of the solution of type I polysaccharide was injected into the same sites. With the same donor's sera a similar series of injections was performed with type III polysaccharide as a control. The results of the passive transfer tests may be summarized as follows:

TABLE II

Immediate skin reactions in passive transfer tests with human serum and capsular polysaccharides

Donor's blood serum	Before treatment with type I anti-serum (type I agglutinins absent)	After treatment with type I anti-serum (type I agglutinins present)
Type I polysaccharide (homologous type)	—	+
Type III polysaccharide (heterologous type)	—	—

In every case where the donor's serum contained type I agglutinins, a positive reaction was produced by the injection of type I polysaccharide. When the donor serum contained no type I agglutinins, no reaction was observed. None of the serum-polysaccharide tests done with the heterologous (type III) polysaccharide was positive. The positive reactions produced by this passive transfer technique were indistinguishable from those seen in the usual skin reaction to polysaccharide.

In both of the above experiments, it was possible to produce the characteristic immediate wheal and erythema skin reaction to type I capsular polysaccharide by merely "sensitizing" the skin

locally by the intradermal injection of homologous antibody. As long as the antibody was present in sufficiently high titer, it seemed to make no difference whether its source was antipneumococcal rabbit serum or the blood serum of patients convalescing from pneumonia after adequate treatment with therapeutic antiserum. The results of both experiments strongly support the view that the Francis skin reaction is due to a local union of antigen and antibody at the site of injection of the polysaccharide.

II The relationship of the titer of circulating antibody to the dermal reaction to capsular polysaccharide

It has been shown by the experiments just described that, in order to produce an immediate wheal and erythema skin reaction by injecting antibody and homologous polysaccharide intradermally at the same site, it is necessary to inject a relatively large amount of antibody. This fact suggests that the titer of antibody in the blood serum of patients with pneumonia may be a determining factor in the production of a positive skin reaction. That specific antibodies may be present in the blood serum at a time when the polysaccharide skin test is negative has been well established (8, 9, 10, 13) but the relation of the titer of antibody to positive skin reactions has never been carefully studied.

In the following experiment the titer of antibody in the blood serum of three patients with type I pneumonia was followed throughout the course of serum therapy by means of mouse protection tests. Blood was drawn before and after each of the hourly injections of therapeutic serum (excluding the first 50,000 units) and at convenient intervals after serum therapy had been discontinued. The results of the antibody studies in two of the cases are depicted graphically in Figures 1 and 2. In all three cases protective antibodies appeared in the blood after the first 50,000 units of therapeutic serum had been given, although the Francis skin test remained negative. Not until the antibody titer had reached a level of one-tenth of a unit or more per cc. did the skin reaction become positive. These data indicate that not only the presence but the titer of type-specific antibodies in the blood serum is an important factor determining the reaction to in-

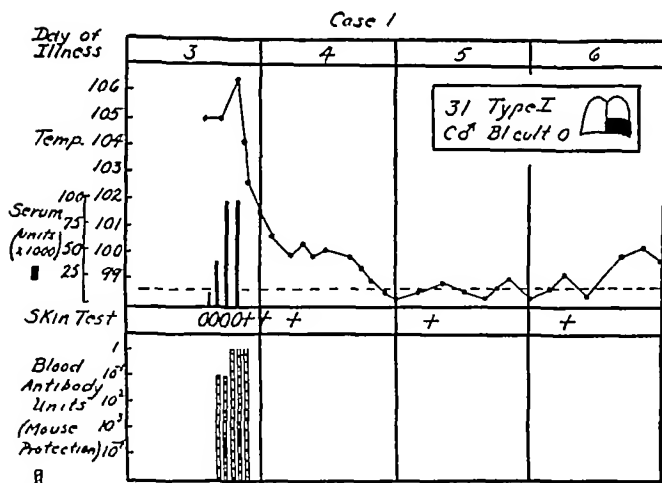


FIG. 1. ANTIBODY CONTENT OF THE BLOOD IN PNEUMOCOCCAL PNEUMONIA TREATED WITH ANTISERUM

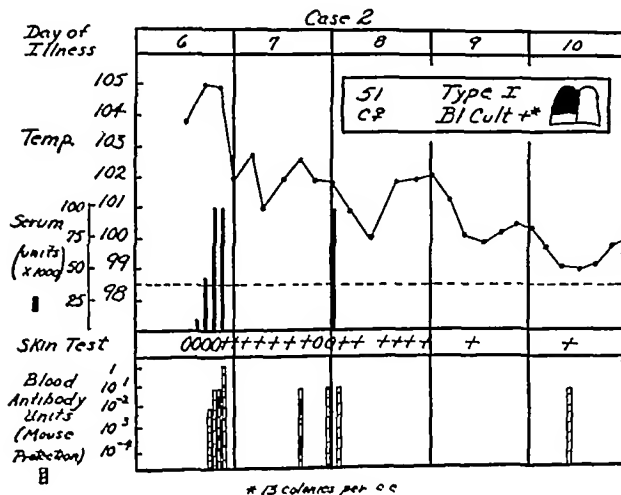


FIG. 2. ANTIBODY CONTENT OF THE BLOOD IN PNEUMOCOCCAL PNEUMONIA TREATED WITH ANTISERUM

tradermal injection of homologous capsular polysaccharide

It will be noticed that in the case summarized in Figure 2 the skin reaction became negative on the second day without any demonstrable decrease in the titer of antibody in the blood at the time that the skin failed to react. This can be explained in one of two ways. Either the mouse protection method as used in these tests was too crude a measure of antibody to detect so slight a decrease as may have occurred, or else, the reactivity of the skin was temporarily depressed so that the amount of antibody in the blood was insufficient to cause the skin to react. As regards the first possibility no further data are available, but the following observations substantiate the view expressed by Francis that the reactivity of the skin to capsular polysaccharide may vary during the course of a pneumococcal pneumonia.

III The reactivity of the skin as a limiting factor in the skin reaction to capsular polysaccharide

In their earliest investigations of the skin reaction to type-specific polysaccharide, Tillett and Francis observed that in fatal cases of pneumonia the skin reaction remained negative in spite of the presence of relatively large amounts of antibody in the blood. Francis noted that a positive reaction does not depend solely upon a high concentration of circulating antibodies, since in certain cases the titer of agglutinins was found to be the same before recovery, when the skin test was negative, as it was later, when the skin reaction had become positive. He concluded that a positive skin reaction to a given polysaccharide depended upon two factors: (1) the presence of homologous type-specific antibodies in the blood serum and (2) the state or reactivity of the skin. Postulating that an increase in the reactivity of the skin was associated with recovery from pneumonia, he suggested that the negative skin reactions encountered in fatal cases were due to a loss of cutaneous sensitivity.

As evidence that an increase in the reactivity of the skin was associated with recovery, Francis cited the experiments of Finland and Sutcliffe (13). They reported that skin tests done with both type I and type II polysaccharides became positive at the same time (approximately the time of crisis)

in patients with type I pneumonia treated with bivalent antiserum (types I and II). The good reason to believe that the blood of a patient suffering from type I pneumonia will acquire an excess of type II antibody before type I, is that a patient is treated with bivalent antiserum containing approximately equal amounts of both antibodies (2, 19). This fact cannot be reconciled with the results of Finland and Sutcliffe's experiments unless it is assumed that the skin reaction to capsular polysaccharide depends upon an increase in reactivity of the skin occurring at a time of recovery rather than upon the appearance of an excess of homologous antibody in the blood.

Since skin tests were done at relatively frequent intervals by Finland and Sutcliffe, their studies of patients treated with bivalent serum were repeated. Two patients with type VII pneumonia, both critically ill with bacteremia, were treated with bivalent horse serum (types V and VII). In both cases the skin test with type V polysaccharide became positive after approximately 1,000 units of type V antibody had been administered, whereas a positive reaction with type VII polysaccharide was not obtained until 1,160,000 units of type VII antibody had been given in one case (see Figure 3) and 1,800,000 units in the other. In a case of type IV pneumonia treated with types IV and VIII serum the skin reaction to type VIII polysaccharide became positive after the injection of 15,000 units of type VIII antibody, in contrast to the type IV skin test which remained negative until 495,000 units of type IV antibody had been administered. In each case much more antibody was needed to produce a positive reaction to the polysaccharide which corresponds in type to the pneumococcus causing the pneumonia. The fact that the skin reaction to polysaccharide of unrelated type became positive in each case long before the patient had received sufficient antibody to bring about recovery indicates clearly that the Francis skin reaction does not depend solely upon an increase in cutaneous reactivity occurring at the time of recovery.

That the reactivity of the skin to polysaccharide may actually change during the course of a pneumococcal pneumonia has never been clearly established. However, the following immunological studies carried out in an unusual case

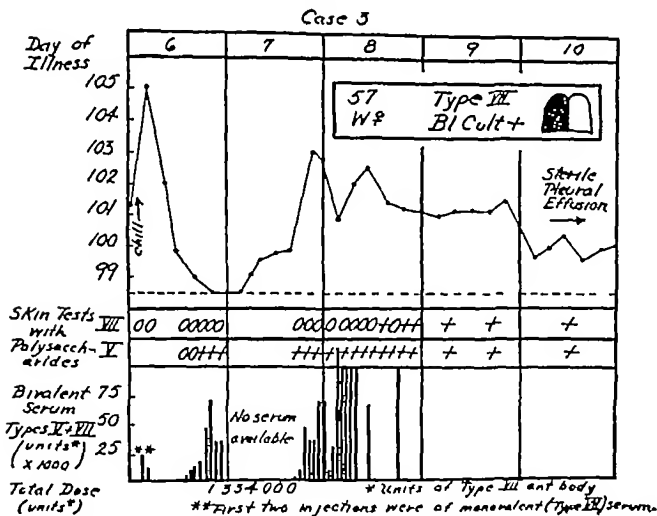


FIG. 3 SKIN REACTIONS TO POLYSACCHARIDES IN PNEUMONIA TREATED WITH BIVALENT ANTISERUM

pneumonia would seem to prove conclusively that the skin may lose its power to react to injections of polysaccharide during the course of a severe pneumonia, even though a large excess of homologous antibody is present in the blood

PROTOCOL

A 50-year-old colored woman was admitted to the hospital on the third day of an attack of lobar pneumonia which involved the left lower lobe. The sputum contained many type III pneumococci typed by direct quellung reaction and after mouse inoculation. No other type of pneumococcus was recovered from the sputum the blood culture was negative. The patient was treated with type III antipneumococcal rabbit serum, and after she had received 160,000 units the skin test with type III polysaccharide became positive and the temperature promptly fell to normal. (See Figure 4) The noticeable improvement in the patient's condition which occurred at this time made it apparent that she had had a crisis. However 12 hours later she experienced a shaking chill the temperature rose to 104 and the skin test became negative. It was thought that she had suffered a spread of the type III pneumonia and serum therapy was resumed. The skin reaction became positive after each of the first two injections of 80,000 units of serum only to become negative within a few hours. From then on it remained negative, in spite of continued intensive treatment with type III serum and in spite of the

fact that agglutinin tests revealed a high titer of type III antibody circulating in the blood. Becoming rapidly worse the patient finally lapsed into coma 12 hours after the chill. Physical examination revealed signs of consolidation of the left upper as well as the left lower lobe (confirmed by x ray). A blood culture taken at the time of the chill, was reported positive 12 hours later and, not type III, but type XVI pneumococci were isolated from the blood broth. The sputum was immediately retyped and was found to contain only type XVI pneumococci. It then became apparent that the patient had developed type XVI pneumonia involving the left upper lobe only 12 hours after recovering from a type III infection of her left lower lobe. Desperately ill, with a heavy bacteremia (22 colonies of type XVI pneumococcus per cc.) the patient was treated with large doses of sodium sulfapyridine intravenously (20). She responded dramatically to this treatment, regaining consciousness in less than 8 hours. Early the following morning 16 hours after the first injection of sodium sulfapyridine, the temperature fell to normal, and thereafter the patient made an uneventful recovery. Meanwhile, during the course of the sulfapyridine therapy the skin test with type III polysaccharide was performed repeatedly. While the patient was critically ill from the type XVI infection, the skin test remained persistently negative. Only after she had regained consciousness and the temperature had fallen to 99 did the skin test with type III polysaccharide again become positive to remain so throughout her convalescence.

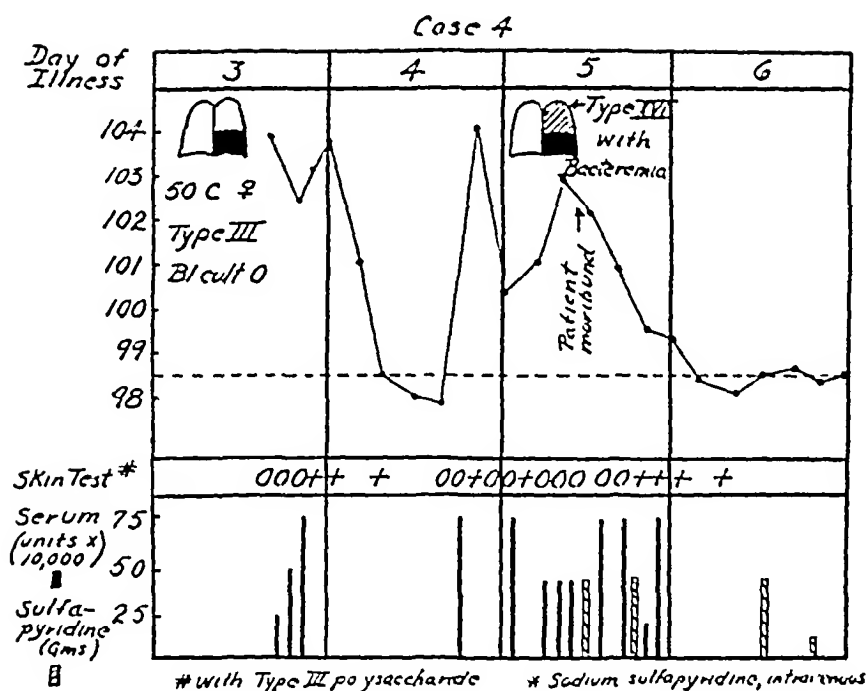


FIG. 4. CHANGE OF REACTIVITY OF SKIN TO POLYSACCHARIDE IN PNEUMONIA DURING RELAPSE CAUSED BY PNEUMOCOCCUS OF A DIFFERENT TYPE

The changes in the skin reaction to type III polysaccharide observed in this case during the course of the type XVI pneumonia cannot be attributed to a decrease in the titer of type III antibody in the blood. Careful examination of both blood and sputum revealed only type XVI pneumococci during the relapse so that there was no bacteriological evidence of a flare-up of the type III infection. Also, intensive therapy with type III antiserum was continued, and it is only reasonable to suppose that the serum treatment, in the absence of an active type III infection, caused a considerable excess of type III antibody to accumulate in the blood. That a high titer of type III agglutinins was present in the blood when the skin test remained negative was shown by agglutination tests. It is apparent that the failure of the skin to react to type III polysaccharide was due to a loss of cutaneous reactivity in some way associated with the "toxemia" of the type XVI pneumonia rather than to a decrease in the titer of type III antibodies in the blood. The loss of cutaneous reactivity was evidently progressive as shown by the fact that the first two injections of serum after the chill caused the skin to revert temporarily to positive, whereas re-

peated injections thereafter failed to produce a positive reaction until finally the type XVI infection had been controlled.

It may be concluded from the observations made upon this patient and upon the patients treated with bivalent antiserum that, although the Francis reaction does not depend solely upon an increase in dermal reactivity occurring at the time of recovery, the skin of patients suffering from pneumonia may lose its power to react to injections of polysaccharide, even in the presence of large amounts of antibody in the blood. This loss of cutaneous reactivity is commonly associated with profound toxemia and, as emphasized by Francis, it usually indicates a poor prognosis.

DISCUSSION

Evidence has been presented that the immediate wheal and erythema skin reaction to capsular polysaccharides of the pneumococcus is due to the local union of antibody and polysaccharide in the skin. A positive skin reaction to the intradermal injection of a given type of polysaccharide has been shown by Francis and others to be invariably associated with the presence of

homologous type specific antibodies in the blood. Cutaneous reactions simulating exactly those seen in the Francis skin test have been produced by injecting homologous antiserum and polysaccharide into the same site of the skin of normal individuals who did not react to injections of the polysaccharide alone. The intracutaneous injection of antiserum 'sensitized' the skin locally to the homologous polysaccharide and the resulting skin reaction was indistinguishable from that caused by similar injections of polysaccharide alone in patients convalescing from pneumococcal pneumonia. Positive reactions were also passively transferred to negative reactors by the usual passive transfer technique. The factor, passively transferred, which caused the positive reaction, was shown to be the type-specific antibody since the skin was sensitized only when the donor's serum contained antibody and reacted only to polysaccharide of the homologous type.

The Francis skin reaction may perhaps be best regarded as the result of a precipitin reaction occurring locally in the skin. It is conceivable that antibody circulating in the blood stream combines with the locally injected homologous polysaccharide and sets up a characteristic dermal reaction. In order that such a dermal reaction be produced two conditions must obtain.

First, there must be sufficient antibody in the blood to react with the 0.1 mgm of polysaccharide injected intradermally and the polysaccharide-antibody reaction must be sufficiently strong to cause the skin to react locally. That enough antibody must be present was shown by the fact that, in order to produce a positive reaction by injecting 0.1 cc. each of antiserum and polysaccharide intradermally at the same site it was necessary to introduce more than one-tenth of a unit of antibody. Likewise patients treated with antiserum did not develop a positive reaction as soon as antibody could be demonstrated in the blood but only after a relatively high titer of antibody had been reached.

Secondly, the dermal reaction must depend in part upon the state of reactivity of the skin. That cutaneous reactivity may be depressed during the course of pneumonia has been shown in the case of type III pneumonia reported above. A similar decrease in the reactivity of the skin to tuberculin and Dick and Schick toxins is known

to occur during the febrile stage of pneumonia and other acute infectious diseases (21). The most logical explanation for the negative skin reactions to polysaccharide observed repeatedly in patients dying from pneumonia, in spite of the presence of large amounts of antibody in the blood would seem to be a loss of cutaneous reactivity.

As a test for the presence of pneumococcal antibody in the blood serum the Francis skin test is extremely crude. Finland and Sutliff (22) have found it less sensitive than either mouse protection or agglutinin tests. Not only is it relatively insensitive but also the end point at which the reaction occurs is variable depending upon the state of reactivity of the skin. Strangely enough it seems to be these two properties which render the skin test with pneumococcal polysaccharide a more reliable guide of prognosis and serum therapy than any of the usual serological tests for antibody (7).

SUMMARY

1 The immediate wheal and erythema skin reaction to pneumococcal capsular polysaccharides which has been observed in patients convalescing from pneumococcal pneumonia is due to a local union of polysaccharide and antibody in the skin as evidenced by the following facts:

(a) Type specific pneumococcal antibody is invariably present in the blood of patients convalescing from pneumonia who react positively to the intradermal injection of capsular polysaccharide. A reaction occurs only when the antibody and polysaccharide are of the same type.

(b) By injecting locally at the same site antiserum and capsular polysaccharide of homologous type an immediate wheal and erythema dermal reaction simulating exactly the Francis skin reaction has been produced in normal individuals who do not react to the polysaccharide alone.

(c) Positive skin reactions to pneumococcal capsular polysaccharide have been passively transferred to negative reactors by previously sensitizing the skin locally with intradermal injections of blood serum obtained from convalescent patients adequately treated with antipneumococcal serum. Evidence is presented that the factor transferred in the donor's blood serum is the type-specific pneumococcal antibody.

2 The skin reaction to capsular polysaccharide depends not upon the mere presence of homologous type-specific antibody in the blood but upon the titer of antibody present. In support of this view it has been shown

(a) that relatively large amounts of antibody must be injected with homologous polysaccharide to produce the characteristic dermal reaction in normal individuals who do not react to the polysaccharide alone, and

(b) that patients with pneumonia, who are being treated with antipneumococcal serum, do not react to polysaccharide as soon as antibody appears in the blood, but only after a relatively large amount of antibody has accumulated

3 The Francis skin reaction is also dependent in part upon the state of reactivity of the skin. It has been demonstrated that positive reactions are not due solely to an increase in cutaneous reactivity occurring at the time of recovery, but that the skin may occasionally lose its ability to react to capsular polysaccharide during the course of severe pneumonia. The loss of cutaneous sensitivity seems to be associated with general toxemia and probably explains the persistently negative reactions observed in patients who die of pneumonia in spite of the presence of appreciable amounts of antibody in the blood

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THE CONTROL OF THE DOSAGE OF ANTISERUM IN THE TREATMENT OF PNEUMOCOCCAL PNEUMONIA. II THE CLINICAL APPLICATION OF THE FRANCIS SKIN TEST

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For many years antipneumococcal serum was administered in relatively small doses at intervals of 8 hours and injections were continued until the fever subsided (1) Such a slow method of giving antiserum necessitated a prolonged period of treatment and patients often succumbed to the pneumonia before they had been given adequate quantities of antibody The importance of administering large doses of serum early in the course of treatment is now well recognized and has recently been reemphasized by Bullowa (2)

It has been suggested that the ideal method of administering antiserum is to give the entire effective therapeutic dose in a single injection. This has been done by certain investigators with apparent success (3), but the method is highly impractical because, for reasons emphasized in a previous report (4) and illustrated by certain of the cases reported in the present study it is quite impossible to calculate even roughly the effective therapeutic dose of antiserum for any given patient with pneumonia To give more than the minimum effective dose only wastes serum and increases the cost of treatment.

Neither the slow method of giving repeated injections of antiserum at intervals of several hours nor the ultra rapid method of attempting to administer the entire effective dose in a single injection is theoretically sound In order to determine the optimum amount of antiserum to be given patients with pneumococcal pneumonia it is necessary to employ some method of controlling the dosage of serum based upon the measurement of type-specific antibody circulating in the patient's blood

Various methods have been advocated in the past none of which has proved to be entirely satisfactory The most widely used has been the microscopic agglutination test introduced by Sabin in 1930 (5) Besides minor technical drawbacks which are inherent in any agglutination method

(4) the test may under certain circumstances, be unreliable as a guide to serum therapy To illustrate this fact the following case report is briefly presented (see Figure 1)

A 51 year-old Russian laborer with type I pneumonia involving the lower lobe of the right lung was admitted to the hospital on the third day of illness. Blood culture showed 50 colonies of type I pneumococcus per cc. The patient was treated with type I antipneumococcal serum and an attempt was made to control the dosage of antiserum by the agglutination method. Serum treatment was discontinued as soon as a definitely positive agglutination reaction was obtained but the temperature remained elevated, and the patient failed to improve in spite of the fact that repeated tests revealed the presence of type I agglutinins in his blood serum. Finally after 4 days, more antiserum was given and the prompt response which followed indicated clearly that insufficient antibody had been given in the first course of treatment.

The agglutination method cannot be relied upon as a guide to serum dosage because of the fact that agglutinins may be detected in the patient's blood serum as in the above case before adequate amounts of antibody have been given (6) Bullowa (7) has attempted to avoid this difficulty by using a roughly quantitative modification of the Sabin method The quantitative modification appears to be more satisfactory but even this method has been found occasionally to be unreliable (9)

A "capsule swelling test" based upon the Neufeld "quellung" reaction (8) has also been used by Bullowa and Sharff (9) to follow the antibody content of the blood of patients receiving anti-pneumococcal serum Although the test was found to be technically simple the authors reported that it was no more reliable than the quantitative microscopic agglutination test, both being positive on one occasion in a case of type II pneumonia when bacteremia was present and the disease terminated fatally

A third method of following serum antibodies

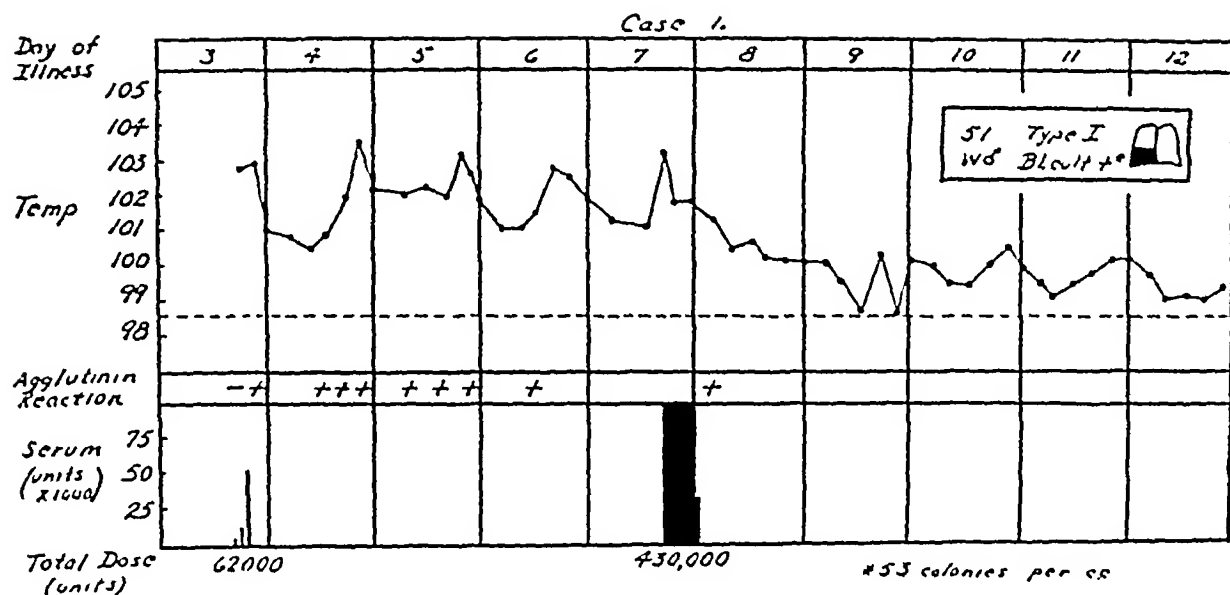


FIG 1 THE PRESENCE OF TYPE-SPECIFIC AGGLUTININS IN THE BLOOD SERUM OF A PATIENT INADEQUATELY TREATED WITH ANTIPNEUMOCOCCAL SERUM

is that introduced by Francis in 1933. The mechanism of the skin reaction to pneumococcal polysaccharides which forms the basis of the Francis skin test has been discussed in a previous publication (4). The present report deals with a clinical study of the skin reaction and its application as a method of controlling serum therapy.

METHODS

Antisera, polysaccharides and skin tests. The antipneumococcal sera, the preparations of type-specific capsular polysaccharides, and the method of performing and reading the skin tests with polysaccharides have been described in a previous report (4).

Selection of cases. All patients entering the hospital with lobar pneumonia and treated with immune serum were included in the present study. The patients treated were suffering from pneumonia caused by pneumococci of types I, II, III, IV, V, VII and VIII. A skin test with the polysaccharide which corresponded in type to the pneumococcus causing the pneumonia was performed in each case before serum treatment was begun. If the skin reaction was positive at this stage, no attempt was made to use the test as a guide to serum therapy, (10).

Control of serum dosage. In all cases where the first skin test was negative, a small initial dose

of antiserum (1,000 to 10,000 units) was given intravenously, followed by injections of from 10,000 to 100,000 units¹ spaced at hourly intervals. The patient's temperature was taken every hour during the period of therapy. A skin test was performed 30 minutes before each injection of antiserum, and as soon as a definitely positive reaction was obtained, the serum therapy was discontinued regardless of the clinical status of the patient. The test was then repeated in one hour and, if still positive, no further therapy was given. Skin tests were performed thereafter at least once a day (and more often in the presence of fever) until recovery was well established. Under no circumstances was serum treatment resumed unless the skin reaction became negative. These rules were adhered to strictly because only by so doing was it thought that the worth of the skin test could be accurately evaluated.

RESULTS

Limitations of the skin test method. Fifty-seven patients with pneumococcal pneumonia were tested with the polysaccharide corresponding in type to the pneumococcus identified in the sputum, at a time when the disease was apparently still progressing, 5 (or 8.8 per cent) reacted posi-

¹ On two occasions larger doses were given in a single injection (see Figure 1 and Figure 2, Case 4).

tively before receiving any antiserum. All 5 patients were treated with immune serum and all recovered. Since the Francis skin test was positive from the start, it obviously could not be used to control the dosage of antibody. Two of these patients also reacted positively to polysaccharides of other types. The blood sera of 2 of the 5 patients were tested before treatment for the presence of type-specific antibody: one by microscopic agglutination tests, the other by the mouse protection method. No antibodies were detected in either serum. The mechanism of these "false positive" reactions is apparently quite different from that of the reaction to homologous polysaccharide which occurs with recovery from pneumococcal pneumonia, since the latter is always associated with the presence of type-specific antibodies in the blood serum (6).

In agreement with these results, MacLeod, Hoagland and Beeson (10) have recently reported that 125 per cent of a series of 104 patients showed positive skin tests before serum had been given. Type specific agglutinins were not present in the blood serum of any of the 4 patients tested among the positive reactors. None of the patients gave a history of a previous pneumococcal infection and in only 1 case was there a history of any form of cutaneous hypersensitivity. The authors were unable to account for the positive skin reactions in these patients but concluded that they were not associated with the presence of circulating antibodies in the blood serum. They also pointed out that in individuals acutely ill with pneumonia the incidence of positive skin reactions is much lower than among normal individuals who are not suffering from pneumococcal infections (11, 12, 13).

It should be clearly understood that if the Francis skin test is to be used as a guide to serum therapy, it must be performed on every patient before serum treatment is begun. The test obviously cannot be used to control serum dosage in patients who react positively from the start. Fortunately, the occurrence of false positive reactions is not sufficiently frequent to handicap the method seriously.

Difficulty was occasionally encountered in reading the skin test, especially in Negro patients. The zone of erythema which surrounds the wheal in a positive reaction is sometimes barely per-

ceptible in the melanotic skin unless viewed under a very bright light. Early in the course of the present study it was found impossible to read the Francis test in one extremely dark Negro patient, and the attempt to control the dosage of serum by means of the test had to be abandoned. It was later learned that the zone of erythema could be easily seen in the skin of even the darkest Negroes if the site of injection was examined under the proper light. Thereafter a 100-watt lamp held close to the skin was used in reading all tests and no further difficulty was encountered.

The skin test as a guide to serum therapy. The skin test with type specific polysaccharides was used to control the dosage of antiserum in the treatment of 51 patients with pneumococcal pneumonia. To simplify presentation the cases may be grouped as follows:

(1) *Uncomplicated pneumonia treated with antiserum.* Twenty-four patients ill with uncomplicated pneumonia responded promptly to serum therapy. In every case the Francis skin reaction became positive shortly before or at the time of crisis. In no case did the temperature reach normal before a positive skin reaction was obtained. The temperature charts of 4 representative patients from the group are shown in Figure 2. It will be noticed that in Case 1 the temperature was approximately 104° when the skin test became positive and serum treatment was stopped. The temperature remained at this level for more than 4 hours before the crisis finally ensued. By using the skin test it was possible to administer antibody rapidly and still conserve antiserum by stopping treatment several hours before crisis. If the temperature chart alone had been used as a guide to serum dosage 200,000 units of antibody would have been wasted. Case 2 illustrates a similar situation. The patient suffered a thermal reaction to type III rabbit serum, the temperature rising to 107°. After 160,000 units of serum had been given however the temperature had fallen to 102.2° and the skin test became positive. During the next 3 hours the temperature rose gradually to 103° but since the skin reaction remained positive, no further treatment was given. At the end of 3 hours a crisis occurred and the patient made an uneventful recovery.

Cases 3 and 4 are included in Figure 2 to em-

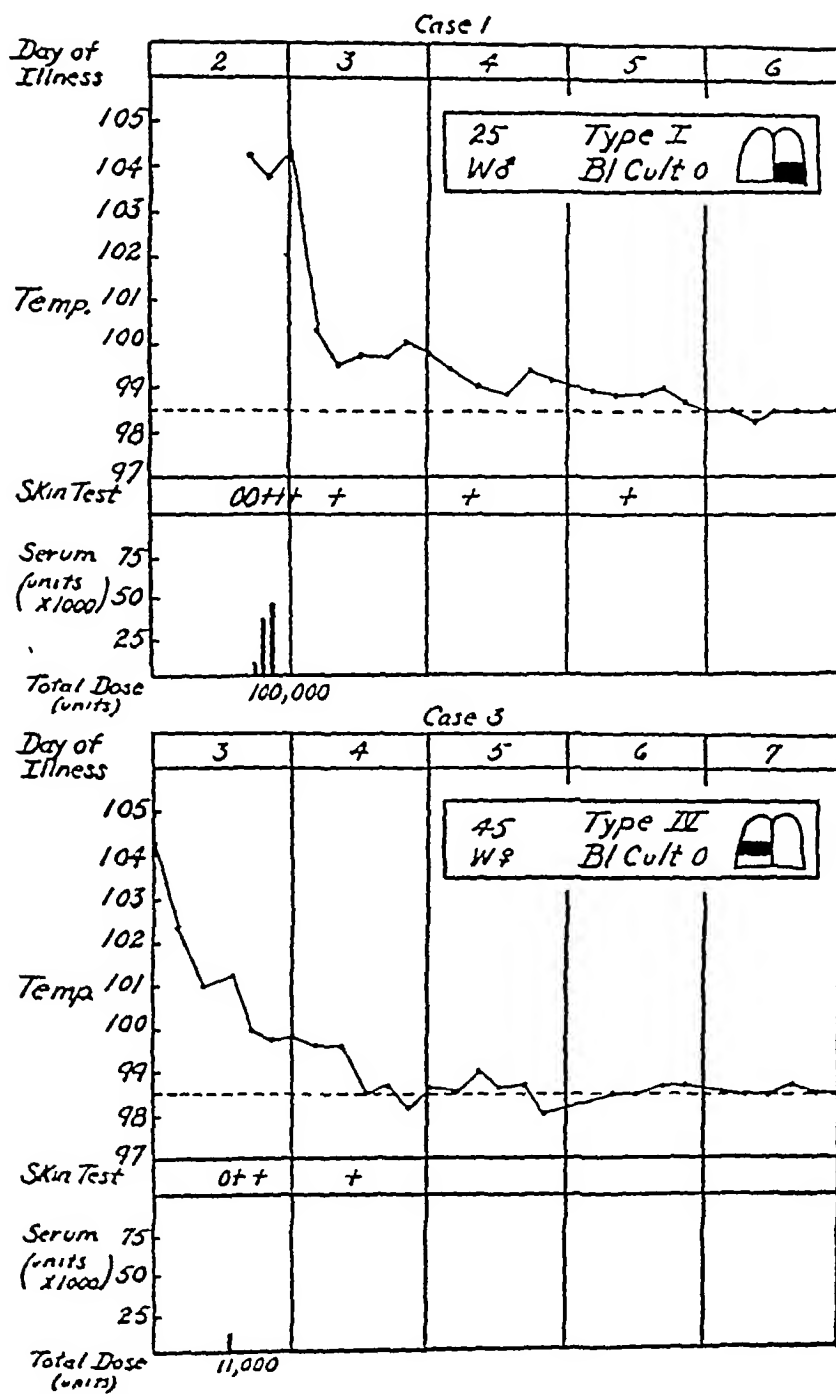
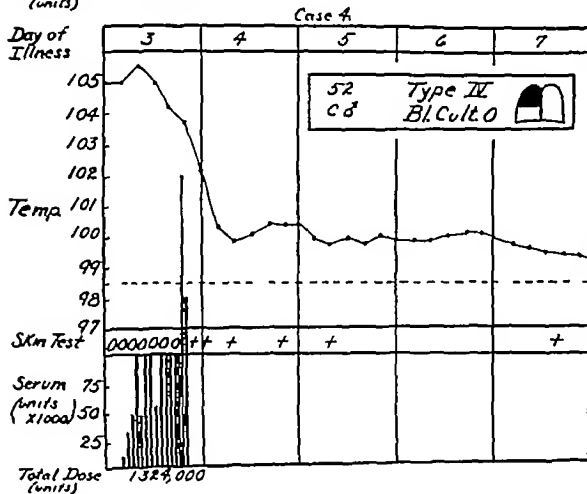
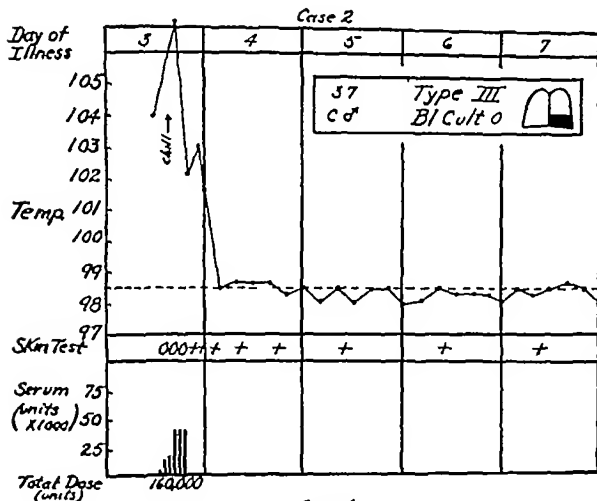


FIG. 2 THE FALCIS SKIN TEST IN UNCOMPLICATED PNEUMOCOCCAL PNEUMONIA TREATED WITH ACTISERLIN

(Figure 2 continued on opposite page)



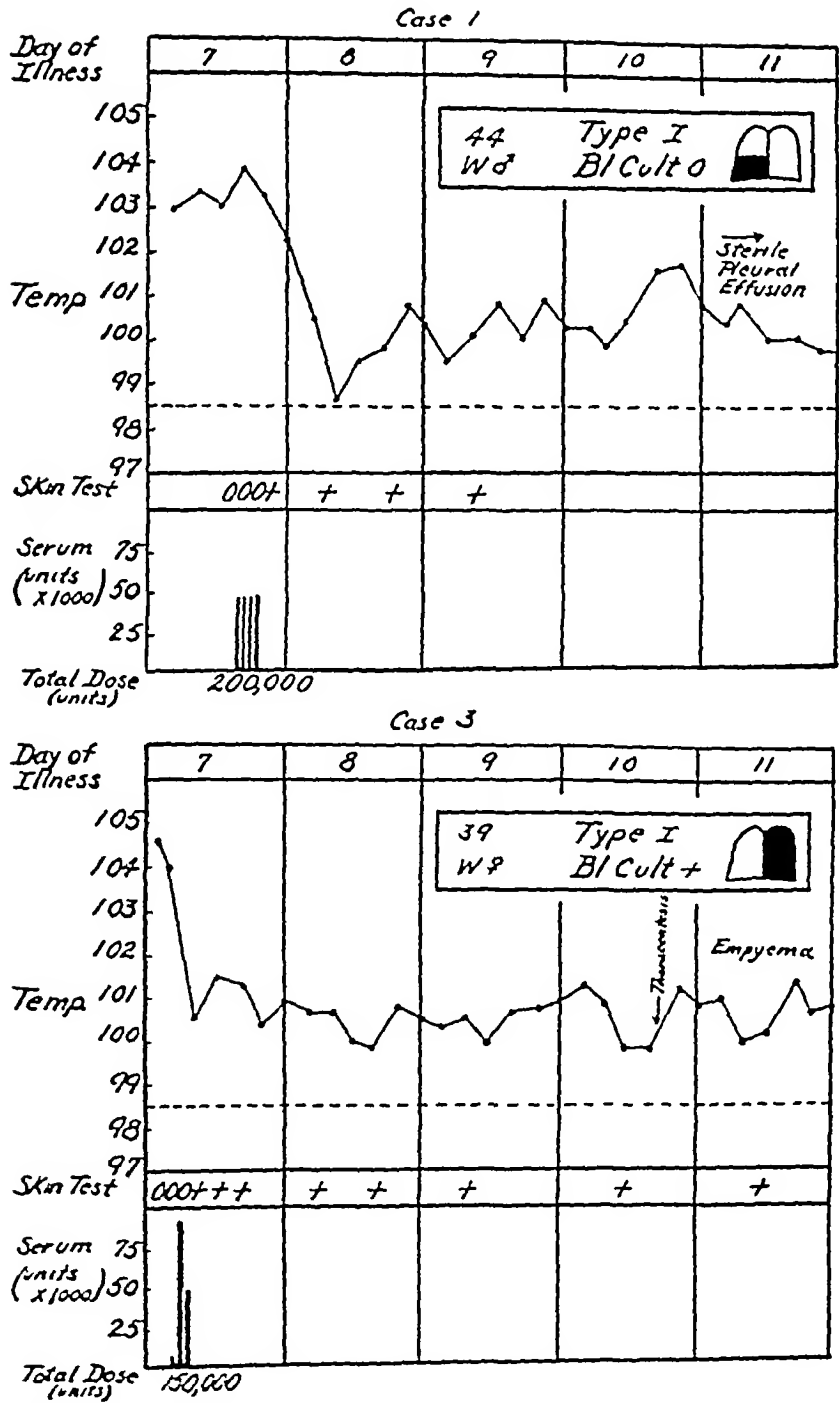
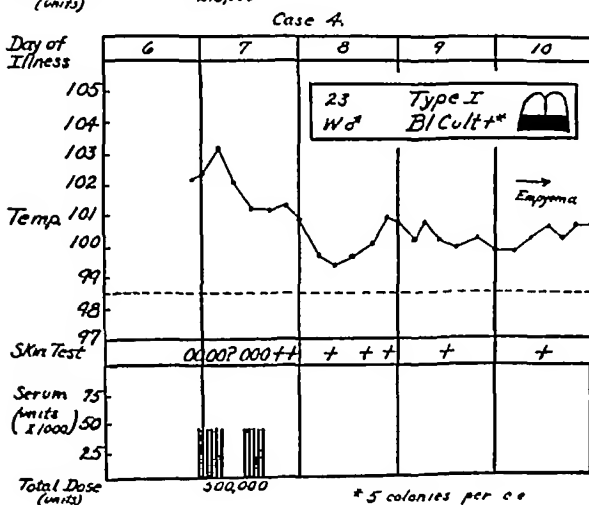
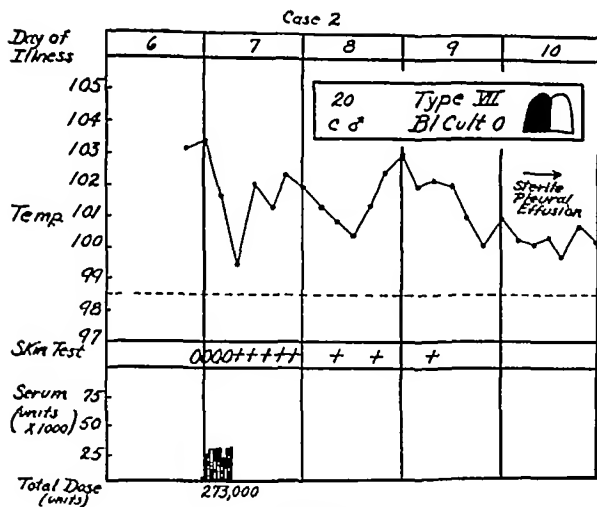


FIG. 4 THE FRANCIS SKIN TEST IN PNEUMOCOCCAL PNEUMONIA TREATED WITH ANTISERUM AND COMPLICATED BY STERILE PLEURAL EFFUSION OR EMPYEMA

(Figure 4 continued on opposite page)



Six patients in the present series developed sterile effusions in the pleural cavity. In every case fever either persisted after the skin test had become positive, or else a secondary rise in temperature occurred after crisis in spite of a persistently positive skin reaction. The positive Francis tests in the presence of continued fever suggested some extrapulmonary complication, and on each occasion this suspicion was confirmed by thoracentesis. Three cases of empyema were also encountered in which the positive skin reaction in the presence of fever suggested the diagnosis. None of the 9 patients was given additional antiserum after the Francis test had become positive, and all 9 recovered. The empyemas were treated by surgical drainage. The results of the skin tests with type-specific polysaccharides in representative cases of pneumonia treated with antiserum and complicated by sterile pleural effusion and empyema are shown in Figure 4.

One instance each of meningitis and endocarditis occurred in the group studied, both are of considerable interest. The first patient (Case 1, Figure 7) showed a positive skin reaction after he had received 300,000 units of type II antibody. A blood culture taken at the time of his admission to the hospital revealed 680 colonies of type II pneumococcus per cc, a second culture, taken after treatment, was sterile. In spite of persistently positive skin tests, the temperature remained elevated until death. Autopsy revealed a purulent meningitis from which type II pneumococci were isolated in pure culture. Since the patient apparently died of an extrapulmonary focus of infection, it is not surprising that his blood serum contained sufficient antibody to maintain a positive skin reaction.

The second patient entered the hospital suffering from type V pneumonia with bacteremia and the physical signs of aortic insufficiency (Case 2, Figure 7). She was given 1,180,000 units of type V antibody and the Francis reaction finally became positive and remained so even in the presence of bacteremia and continued fever. After nearly 3 weeks in the hospital the patient died and at postmortem examination there was found an

serum due perhaps to its relatively small molecular size (15-19) will penetrate the pleura. In spite of this fact the use of antipneumococcal rabbit serum instead of horse serum has resulted only in the occurrence of empyema (20).

acute bacterial endocarditis involving the aortic valve from which was cultured a type V pneumococcus. Although an old organizing pneumonia was present in the left lower lobe, no pneumococci could be isolated from the lungs. It is noteworthy that in this case the skin reaction to polysaccharide was positive at a time when bacteria were cultivated from the blood stream. According to evidence available at the present time the Francis skin reaction does not become positive in the presence of bacteremia unless the pneumonia is complicated by a pneumococcal endocarditis. Antibodies can seldom be detected in the blood when the pneumococcal infection in the lung is advancing with sufficient rapidity to invade the blood stream. On the other hand, it is well known that the antibody content of the blood serum of both patients and experimental animals with pneumococcal endocarditis may be relatively high (16, 21), and it is undoubtedly this fact which explains the positive skin reactions observed in pneumonia complicated by pneumococcal lesions of the heart valves.

It may be concluded from the above results that the Francis skin reaction not only serves as an important aid in the diagnosis of the common complications of pneumonia but also makes it possible to avoid wasting antiserum in cases in which a secondary rise in temperature or a prolonged fever is a disturbing feature.

(4) *Relapse of pneumonia following inadequate treatment with antiserum.* Secondary hyperpyrexia occurring in serum-treated pneumonia is not uncommonly due to a spread of the pneumonic process following inadequate treatment rather than to the common complications just discussed. The immediate recognition of a flare-up of the pneumococcal infection in the lung is of the greatest importance since the progressing lesion can often be checked only by additional therapy. It has been found that, when treatment has been inadequate, the skin reaction to polysaccharide does not remain positive. In 6 instances in the present series of cases the skin reaction reverted to negative after having become positive during serum therapy. One patient never developed a persistently positive skin reaction in spite of continued serum treatment and finally died (Case 2, Figure 8). A secondary rise in temperature occurred in each of the 5 remaining cases at

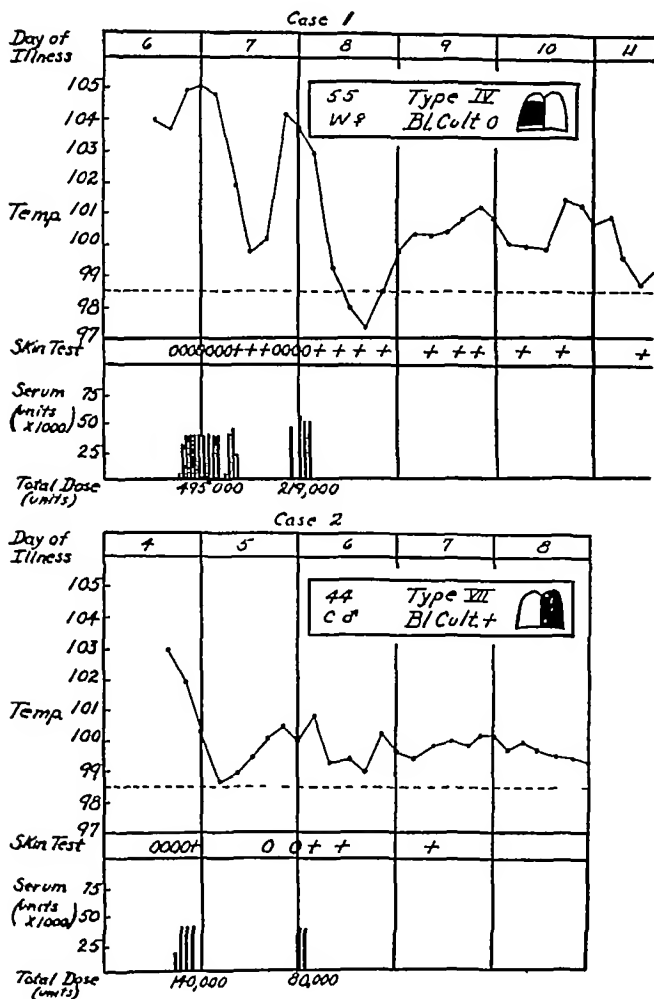


FIG. 5 THE FRANCIS SKIN TEST IN PNEUMOCOCCAL PNEUMONIA REQUIRING MORE THAN ONE COURSE OF TREATMENT WITH ANTISERUM

the time when the skin reaction became negative, and all 5 patients were given more antiserum. Following the second course of treatment the skin reaction again became positive and the fever subsided. Four of the 5 patients recovered, the clinical course of the pneumonia in 2 of these cases is summarized in Figure 5. The fifth patient finally died in uremia (Case I, Figure 8), and his record will be referred to presently.

These results would seem to confirm the conclusion reached by Francis that a negative skin reaction invariably indicates further serum therapy. By the use of the skin test with type-specific polysaccharides it is possible to distinguish between fever due to an extrapulmonary complication and that due to a progression of the pneumonic infection in the lung. If the fever is caused by the former, the skin reaction remains positive, when due to the latter, it becomes negative. To be able to make this distinction is of practical significance, for although the usual complications of pneumonia are unaffected by continued serum treatment, advancing pneumonia should be controlled immediately by further specific therapy.

(5) *Patients with pneumonia who failed to respond to treatment with antiserum.* Six patients among the 51 studied failed to survive in spite of intensive treatment with antipneumococcal serum. Their hospital records are summarized in Figures 6, 7, and 8. The first 2 patients were critically ill with bacteremia when admitted to the hospital and neither received sufficient antiserum during the short period of treatment to influence the course of the pneumonia. The Francis skin test never became positive in either case and both patients died within 20 hours. The third and fourth patients (Figure 7) died with pneumococcal meningitis and endocarditis respectively, the essential features of their illness have already been discussed. The last 2 cases are of particular interest since in both the skin reaction became positive after treatment, although both patients died and neither seemed to be suffering from any form of pneumococcal complication. The first patient, 68 years of age (Figure 8, Case 1), entered the hospital on the fourth day of a type I pneumonia with bacteremia. After he had been given a total of 650,000 units of type I antibody, his Francis skin test remain-

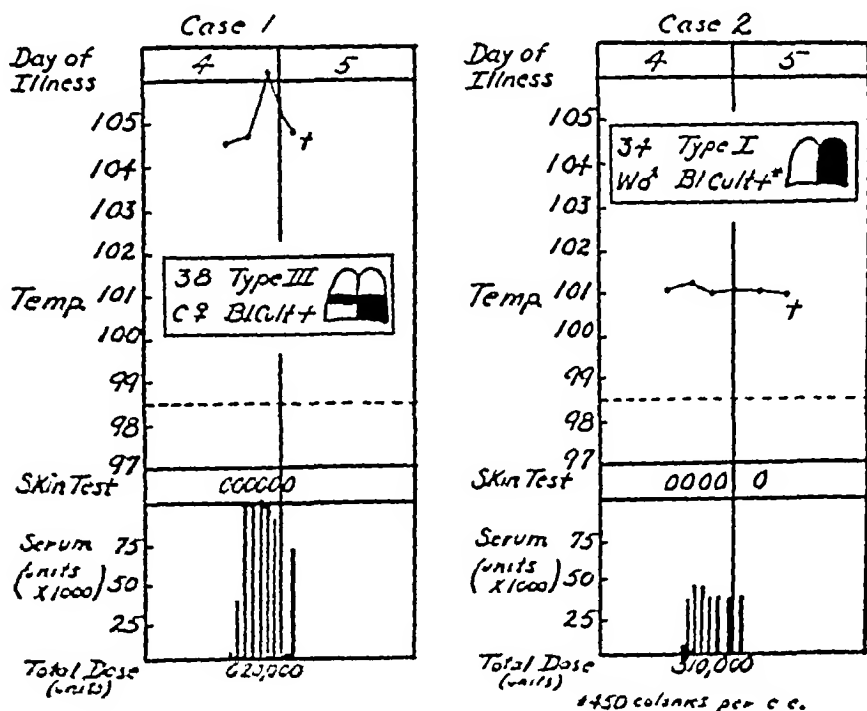
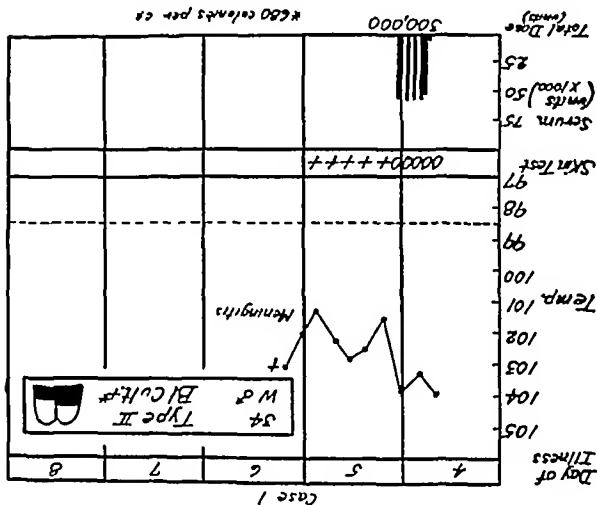
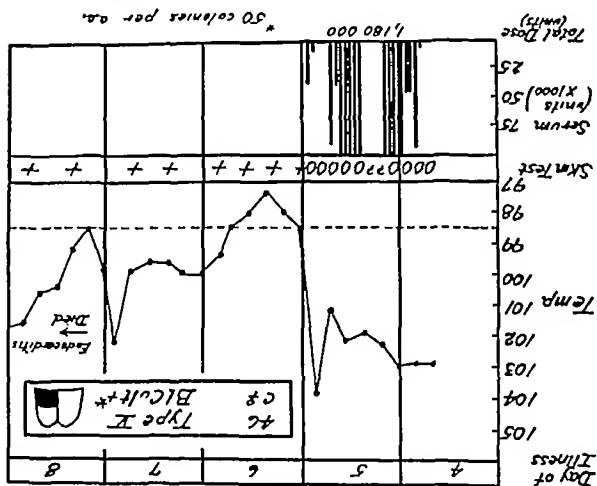


FIG. 6. THE FRANCIS SKIN TEST IN FATAL PNEUMOCOCCAL PNEUMONIA TREATED WITH ANTISERUM.

FIG. 7 THE FRANCIS SKIN TEST IN FATAL PNEUMOCOCCAL PNEUMONIA TREATED WITH ANTISERUM AND COMPLICATED BY MENINGITIS OR ENDOPNEUMONITIS



positive, his temperature fell to 100° and he seemed to be making a good recovery. He was, however, a diabetic with marked hypertension and he finally developed signs of uremia and died on the eighth day of illness. Careful examination of the lungs at autopsy revealed that no pneumococci were present in the alveolar exudate, and the cultures from both lungs and from the blood were sterile. The terminal hyperpyrexia suggested a spread of the pneumonia in spite of the positive skin tests, but the findings at autopsy indicated that the pneumococcal infection had been well controlled and that the patient probably succumbed to the complicating uremia. The second patient was 81 years of age (Figure 8, Case 2), and although her skin test became positive several times following serum therapy, it reverted to negative after a few hours on each occasion and remained negative at the time of death. Terminally, the non-protein nitrogen rose to 160 mgm per cent and, as in the previous case, uremia seemed to be an important factor in the final outcome. Autopsy, however, showed that the pneumonia had not been completely controlled since viable pneumococci were still present in large numbers in the lungs.

Even in the fatal cases of pneumonia the Francis skin test proved to be a reliable guide to serum therapy. Only when death was apparently due to some complication, which would not have been benefited by further serum treatment, did the skin reaction remain positive. Patients dying of pneumonia which was obviously uncontrolled by antibody therapy showed negative skin reactions at the time of death.

pneumococcus involved, the extent of the pulmonary lesion, and the presence or absence of bacterial pregnancy, or a serious complicating disease (2). Although these criteria determine roughly the amount of antiserum the average patient needs, they cannot be applied to any individual case, and therefore are of limited practical value.

During the past year, sulfapyridine has been found to be a very effective drug for control of pneumococcal infections. Although the use of sulfapyridine in the therapy of pneumonia undoubtedly decreases the need for type-specific antiserum, it has already been shown (14) that not all patients with pneumococcal pneumonia will respond to treatment with the drug alone. In the drug-refractory cases immune serum should be administered as an adjunct to chemotherapy, and it is only reasonable to suppose that the doses of antiserum needed may be quite different from those required for comparable patients treated with serum alone. The accepted criteria for estimating the average effective therapeutic dose of antibody are based upon past experience with antipneumococcal serum and probably do not apply to patients who have previously been treated with sulfapyridine. It is doubly important, therefore, that some method of controlling the dose of immune serum be employed in the treatment of patients receiving both immune serum and chemotherapy.

The Francis skin test may be regarded as a relatively crude method of detecting the presence of type-specific antibodies in the patient's blood. Circulating antibodies can invariably be demonstrated by standard serological methods while during the course of pneumonia the skin re-

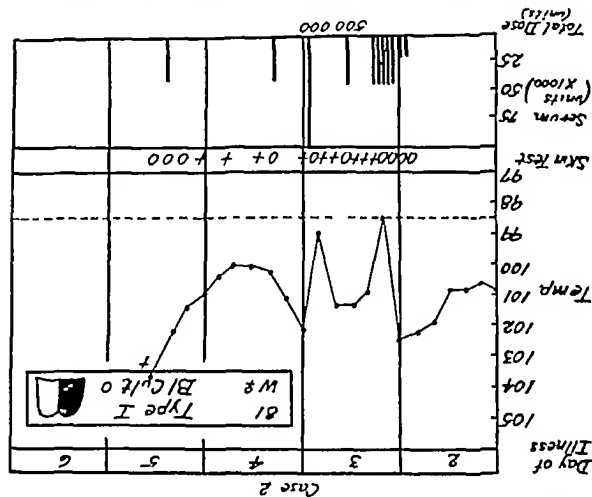
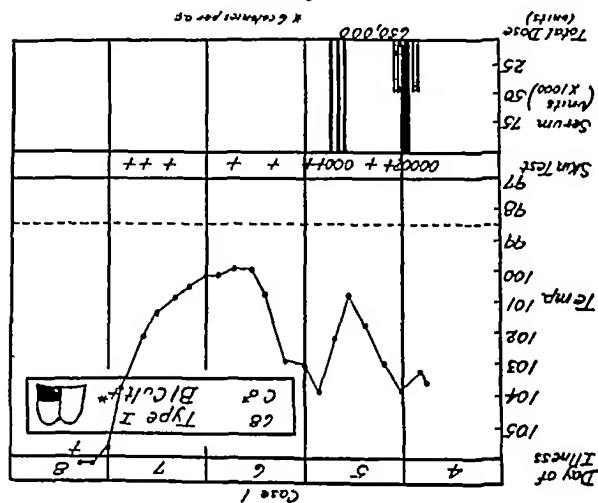


Fig. 8. The Francis Skin Test in Fatal Pneumococcal Pneumonia Complicated by Renal Insufficiency

or during crisis in every case where recovery occurred without complication

(2) All patients whose Francis skin tests became positive and remained positive recovered without further specific therapy except 2 patients who died of pneumococcal meningitis and endocarditis respectively

(3) Every patient who failed to develop a positive skin reaction died

(4) Once positive, the skin reaction remained so in all but 6 cases. Four patients reacted positively after further treatment and recovered promptly, 2 died, one never again developing a persistently positive reaction in spite of continued therapy, the other dying in uremia after the skin reaction had become positive. Autopsy performed upon the last patient revealed that the pneumonia had been well controlled as evidenced by the fact that no viable pneumococci could be recovered from the lungs

(5) Among 6 patients who died, 3 showed positive reactions shortly before the time of death. One was the patient just referred to who died in uremia, the other 2 died with meningitis and endocarditis

(6) In all cases where fever persisted in the presence of a positive skin reaction there was subsequently demonstrated either a sterile pleural effusion or an extrapulmonary pneumococcal infection of the pleura, meninges, or endocardium. Such complications are known to be little affected by continued serum therapy

It is concluded from the results of the present study that the Francis skin test serves as a reliable aid in determining the optimum amount of antibody needed in the treatment of pneumococcal pneumonia. Its use not only makes it possible to treat patients intensively without wasting antiserum, but also aids in the diagnosis of complications and gives valuable information regarding prognosis. The test has been found to be a satisfactory guide to the dosage of antibody in the treatment of patients given both serum and sulapyridine as well as those given serum alone.

SUMMARY

1. Fifty-one patients with lobar pneumonia caused by pneumococci of types I, II, III, IV, V, VII, and VIII were treated with antipneumo-

coccal serum. The dosage of antibody administered in each case was controlled by frequent skin tests with the homologous pneumococcal polysaccharide (Francis skin test)

2. Five patients reacted to the polysaccharide before antiserum had been given. In none of these cases could the Francis skin test be used as a guide to serum therapy

3. The amount of antibody required to control the pneumonia in the cases studied varied from 11,000 to 1,983,000 units

4. Use of the Francis skin test made it possible to treat each patient intensively without wasting antiserum

5. In every case in which a crisis occurred the Francis skin reaction became positive several hours before or during the fall in temperature

6. The skin test served as a valuable aid in the early diagnosis of the complications of pneumonia. In every case in which fever persisted in the presence of a positive skin reaction there was subsequently demonstrated pleurisy with effusion, empyema, meningitis or endocarditis

7. No patient who failed to develop a positive skin reaction survived the pneumonia. Three patients who died reacted positively shortly before death, 2 died of pneumococcal complications, and the third died of uremia, no evidence of active pneumonia being found at autopsy

8. The Francis skin test was also found to be of value in determining the optimum dosage of antibody in the treatment of patients who had previously received sulapyridine

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THE OCCURRENCE OF METHEMOGLOBINEMIA DURING SULFANILAMIDE THERAPY

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The presence of methemoglobin, more rarely sulfhemoglobin, in the blood of patients treated with sulfanilamide has been reported by many investigators (1 a-f). Evelyn and Malloy (2) using their photoelectric colorimeter found both these pigments but felt they were not responsible for the "cyanosis." Wendel (3), using his visual spectroscopic method (4), found methemoglobin and (occasionally) sulfhemoglobin. On the other hand, Marshall (5) Posner (6a) in many instances, Chesley (6b) and some occasional observers have failed to find the characteristic band of methemoglobin.

A little spectroscopic experimentation with pure methemoglobin will speedily convince anyone that the light absorption characteristics of methemoglobin renders visual detection of the band at 630 $m\mu$ rather difficult in the presence of a large excess of hemoglobin. Even Wendel's ingenious method (4) does not eliminate the difficulty of finding this band. If the personal visual factor might be eliminated, incontrovertible evidence might be obtained.

This is possible with the Hardy recording spectrophotometer (7) which accurately draws a curve showing the light transmission of a sample from 400 to 700 $m\mu$. Typical curves of the blood of patients receiving sulfanilamide are reproduced (Figure 1) with normal controls. The prominent depression at 630 $m\mu$ is readily seen. In one case a depression at 620 $m\mu$ is also seen indicating the presence of sulfhemoglobin.

In these patients from 10 to 18 per cent methemoglobin was estimated to be present by using Beer's and Bouguer's laws and absorption coefficients obtained on pure solutions by the Hardy recording spectrophotometer (Table I).

Inasmuch as sulfanilamide *in vitro* does not produce methemoglobin the mechanism of its formation *in vivo* remains unexplained. Further ex-

TABLE I
Absorption coefficients

$\lambda m\mu$	Hemoglobin	Methemoglobin	Colored residue	Blue substance
700	0.0825	0.114	8.64	24.1
660	0.0862	0.472	10.8	56.8
630	0.133	2.32	17.6	75.0
620	0.179	2.18	19.1	80.2
600	0.566	1.98	21.9	89.1
580	7.14	2.40	24.2	93.9
560	5.83	2.52	25.9	92.9
540	8.60	3.70	26.7	87.2
520	4.12	4.75	27.1	78.1
500	3.30	5.57	27.8	68.5
480	4.15	5.00	29.4	59.8
460	6.82	5.69	32.1	53.6
440	16.12	11.5	36.5	49.6

Transmission curves were obtained with solutions of each substance.

These coefficients were calculated after using the equation

$$\log \frac{100}{\% \text{ transmission}} = kcd.$$

k = absorption coefficient

c = concentration of the solution—1 gram per 100 cc. for hemoglobin and methemoglobin

d = thickness of cell in cm.

periments showed that when the blue derivative (8) (a reversible oxidation reduction system formed by the aerobic ultra violet irradiation of dilute aqueous sulfanilamide (9)) is added to hemoglobin, methemoglobin is formed² (Figure 2). Using the accurate Hardy recording spectrophotometer and applying Beer's and Bouguer's laws we analyzed the resulting solution. Assuming that hemoglobin and methemoglobin were the only colored components, the transmissions at 12 different wavelengths from 460 to 700 $m\mu$ were calculated (Table II, Column B). These do not agree with the experimental values (Table II, Column A) and indicate the presence of another colored component. When a third component, the brown solution resulting from the spontaneous

² "It is interesting to note, however that the colored derivatives of sulfanilamide described (by Ottenberg and Fox (8)) convert hemoglobin to methemoglobin *in vitro*." Wendel (3)

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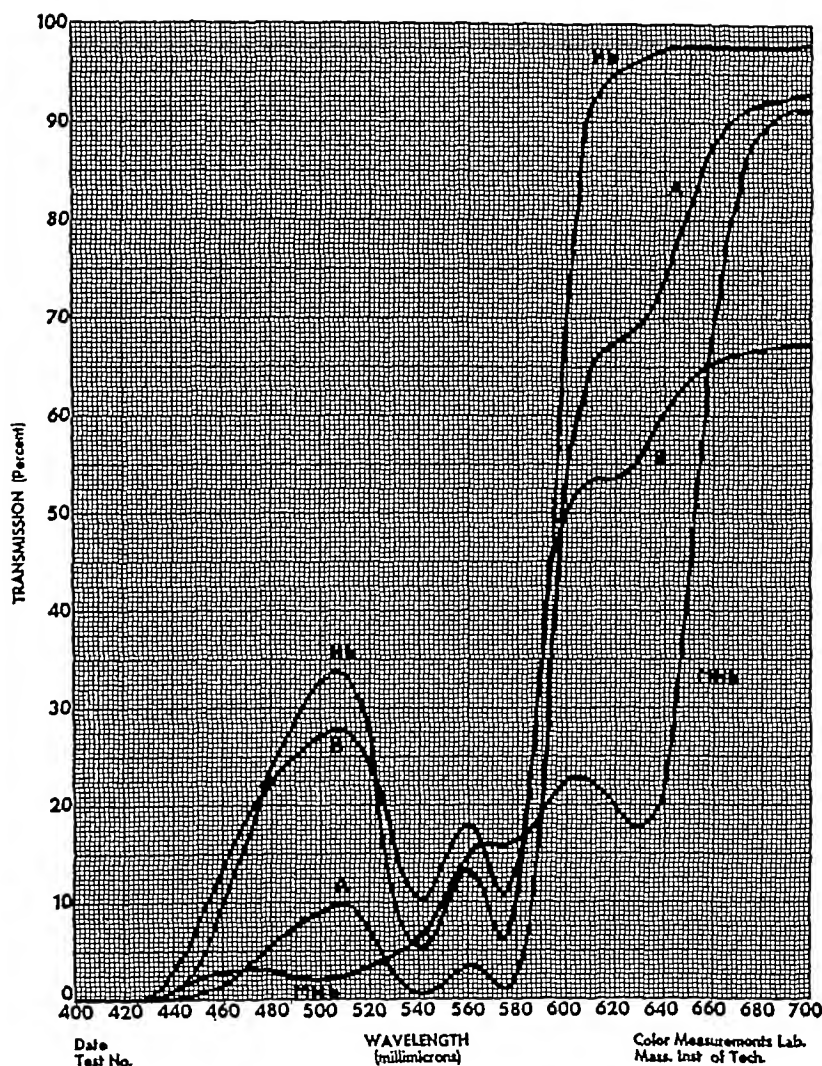


FIG. 1 CURVES OF WASHED, LAKED HUMAN ERYTHROCYTES DRAWN BY HARDY RECORDING SPECTROPHOTOMETER (pH—7.1)

Hb is the curve of 1/100 dilution of blood of normal subject—unaltered hemoglobin solution with characteristic bands at 541 and 573 $m\mu$

MHb is the curve of 100 per cent methemoglobin (same concentration as Hb) with prominent band at 630 $m\mu$

A is typical curve of patients' blood during sulfanilamide therapy (diluted 1/50) showing unmistakable prominence at 630 $m\mu$ caused by methemoglobin and general depression from 660 to 700 $m\mu$ (possibly due to a colored oxide of sulfanilamide)

B is curve of patients' blood during sulfanilamide therapy (diluted 1/100) showing prominence at 620 $m\mu$ caused by sulfhemoglobin and general depression from 660 to 700 $m\mu$ (possibly due to a colored oxide of sulfanilamide)

reduction in air of the blue oxidizing solution, is introduced into the calculations, the predicted transmission values (Table II, Column C) show significantly better agreement with the experimental values (Table II, Column A)

Similar calculations with the data of the curves of patients' blood showed good agreement (Table III) between the observed curve and one predicted on the basis of two components hemoglobin and methemoglobin in the region from

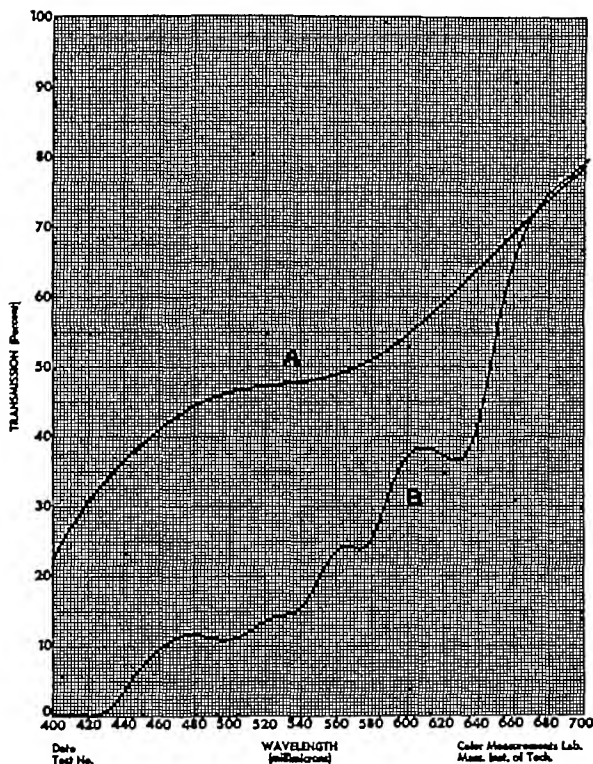


FIG. 2. FORMATION OF METHEMOGLOBIN BY PHOTO-OXIDATION PRODUCT OF SULFANILAMIDE

B is curve after reaction between the blue product of irradiation of sulfanilamide and human hemoglobin.
A is curve of brown solution resulting from spontaneous reduction in air of the blue oxidizing solution

460 to 600 $m\mu$. In this region light absorption by hemoglobin is very great, obscuring small deviations. But in the red region beyond where hemoglobin absorption is minimal, there was demonstrated a small residual absorption. This might be due to any brown substance* such as the brown reduced form of the blue oxidation product (9)

* An unidentified "foreign pigment" is just now reported in normal rats receiving sulfanilamide (10) and Harris and Michel (11) have also observed an extraneous absorption at 670 $m\mu$.

This effect however was too slight to influence the gross appearance of blood. These observations suggest that *in vivo* sulfanilamide is oxidized to the blue derivative which in turn oxidizes hemoglobin to methemoglobin and is itself reversibly reduced. Although this residual absorption indicates in addition to hemoglobin and methemoglobin the existence of a third colored component and while our spectrophotometric findings are consistent with our interpretation equally satisfactory fit of the curves might per-

TABLE II
Hemoglobin—blue substance reaction

$\lambda m\mu$	Experimental A $\log \frac{1}{T}$	Calculated B $\log \frac{1}{T}$	Calculated C $\log \frac{1}{T}$
700	0 118	0 024	0 118
660	0 211	0 094	0 182
630	0 455	0 454	0 455
620	0 460	0 428	0 460
600	0 478	0 396	0 478
580	0 699	0 603	0 638
560	0 693	0 702	0 656
540	0 886	0 885	0 827
520	0 886	1 00	0 876
500	0 959	1 15	0 959
480	0 943	1 05	0 931
460	1 04	1 23	1 07

Experimental A—obtained from spectrophotometer curve
Calculated B—on basis of 91 0 per cent methemoglobin and 9 0 per cent hemoglobin

Calculated C—on basis of 10 8 per cent hemoglobin, 79 7 per cent methemoglobin and 9 5 per cent colored residue

Italicized figures are reference points used in computation

TABLE III
Values of $\log 1/T$ for sulfanilamide-treated patients

$\lambda m\mu$	Patient 1			Patient 2			Patient 3		
	A experi- mental	B cal cu- lated	C cal cu- lated	D experi- mental	E cal cu- lated	F cal cu- lated	G experi- mental	H cal cu- lated	I calcu- lated
700	0 032	0 025	0 038	0 024	0 021	0 024	0 0355	0 023	0 0364
660	0 052	0 046	0 052	0 033	0 032	0 033	0 056	0 047	0 059
630	0 159	0 159	0 159	0 090	0 090	0 090	0 177	0 177	0 177
620	0 169	0 161	0 164	0 100	0 098	0 098	0 180	0 176	0 180
600	0 268	0 237	0 245	0 197	0 184	0 185	0 260	0 236	0 250
580	1 68	1 74	1 80	1 64	1 75	1 75	1 59	1 50	1 56
560	1 47	1 45	1 40	1 444	1 440	1 446	1 29	1 26	1 32
540	2 16	2 14	2 17	2 15	1 92	2 12	1 93	1 86	1 92
520	1 16	1 19	1 20	1 08	1 093	1 095	1 09	1 09	1 10
500	1 05	1 05	1 05	0 92	0 92	0 92	0 99	0 99	0 99
480	1 24	1 21	1 21	1 12	1 11	1 11	1 14	1 11	1 13
460	1 80	1 85	1 87	1 70	1 75	1 76	1 69	1 66	1 69

Column A—Values of $\log 1/T$ from experimental curve

Column B—Values calculated on basis of 80 2 per cent hemoglobin and 19 8 per cent methemoglobin

Column C—Values calculated on basis of 82 3 per cent hemoglobin, 17 4 per cent methemoglobin and 0 314 per cent colored residue

Column D—Values from experimental curve

Column E—Values calculated on basis of 90 3 per cent hemoglobin and 9 7 per cent methemoglobin

Column F—Values calculated on basis of 90 6 per cent hemoglobin, 9 3 per cent methemoglobin and 0 096 per cent colored residue

Column G—Values of $\log 1/T$ from experimental curve

Column H—Values calculated on basis of 74 3 per cent hemoglobin and 25 7 per cent methemoglobin

Column I—Values calculated on basis of 78 0 per cent hemoglobin, 21 4 per cent methemoglobin and 0 573 per cent colored residue

Italicized figures are reference points used in computation

haps be obtained on the basis of different assumptions as to the nature of the pigments present. Our interpretation is consistent with the findings but cannot be considered as established by our data.

Further evidence that *in vivo* sulfanilamide is oxidized is provided by Dr Sanford Rosenthal (12) who reported indications of hydroxylamine sulphonamide, an oxidation product, in the urine of sulfanilamide-treated animals and patients. Preliminary experiments by one of us (C L F) indicate that the Rosenthal test (13) is positive in the methemoglobin forming colored irradiation products and negative in the unadsorbed colorless residual solution which does not form methemoglobin. The data are correlated with the mode of action of sulfanilamide (14).

SUMMARY

1 Objective evidence in the form of curves drawn by the Hardy recording spectrophotometer is presented showing the occurrence of methemoglobin and sulfhemoglobin in the blood of sulfanilamide-treated patients.

2 An explanation for the occurrence of methemoglobin in the blood of sulfanilamide-treated patients is provided by the demonstration *in vitro* of the conversion of hemoglobin to methemoglobin by certain oxidation products of sulfanilamide.

3 Spectrophotometric evidence for the occurrence *in vivo* of this reaction is adduced.

We are greatly indebted to Professor George S Forbes for his valuable suggestions and to Dr Reuben Ottenberg for helpful criticism. We thank the staff of the Color Measurement Laboratory at the Massachusetts Institute of Technology and the House Staff of the Children's Hospital, Boston, for their cooperation.

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EFFECT OF DISEASE OF THE LIVER AND BILIARY TRACT UPON THE PHOSPHATASE ACTIVITY OF THE SERUM

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The present study deals with the influence of common affections of the liver and biliary tract upon the level of serum phosphatase activity in the adult. It is an analysis of our observations during the period 1933 to 1939 on those patients with involvement of the liver or biliary tract in whom the diagnosis could be proven by exploration or necropsy or when insusceptible of proof (as in the hepatitides), could be established with reasonable certainty by clinical methods. Some 350 cases satisfied these criteria: 79 adults with proven obstruction of the common bile duct, 75 cases of hepatitis of indeterminate etiology ("catarrhal" jaundice), 39 cases of jaundice following exposure to known hepatotoxic drugs, 15 cases of hemolytic jaundice, 45 patients with proven cirrhosis of the liver, exclusive of biliary cirrhosis, 46 patients with proven neoplastic involvement of the liver, 10 patients with proven liver abscess, 10 cases of proven chronic passive congestion of the liver without significant "cardiac" cirrhosis, and 29 cases of miscellaneous disorders of the liver or biliary tract. The data in 123 of these patients have been reported elsewhere (1). We record here observations made subsequent to that report together with a study of the distribution of values in our total experience.

On the basis of these empirical clinical data we have attempted to define the clinical usefulness and limitations of the determination of serum phosphatase activity as applied to diseases of the liver and biliary tract. Three such applications of the method appear to be of promise: (1) as a supplementary aid to the clinical differential diagnosis of the several types of jaundice; (2) as an index to certain complications following surgical procedures on the biliary tract; (3) as a rela-

tively early indicator of metastases in patients known to have malignant tumors.

It may be stated at the outset that our data do not wholly support Roberts' disputed claim that by determining the phosphatase activity of the serum "toxic infective and catarrhal jaundice may be readily distinguished from jaundice of the obstructive type" (2). We find, as have most previous investigators (3) that while patients with obstructive jaundice show appreciable elevations in serum phosphatase activity with reasonable consistency, increased serum phosphatase levels of the same order may be observed also in some patients with hepatitis. Whether or not the overlapping of values in the several types of jaundice is sufficient to invalidate the serum phosphatase determination as a practical aid in differential diagnosis is the crux of the problem under consideration and the chief impetus for our studies. These studies have led us to believe that, in general, the determination of serum phosphatase activity affords evidence of limited but definite value in the major practical problem presented by patients with jaundice: the decision between surgical intervention ("surgical jaundice") and conservative management ("medical jaundice"). This usefulness is, of course, contingent upon the recognition of specific deficiencies in the method to be pointed out later and upon a general appreciation of the limitations of any one means of investigation in so complex a phenomenon as jaundice. Reproducibility in technique of the serum phosphatase determination is another essential since application of the method depends upon a comparison of levels of phosphatase activity.

We have attempted further, to derive such generalizations regarding possible mechanisms regulating the serum phosphatase level in hepatic disease as would seem to be justified by the consistency of our data. Obviously, uncontrollable

¹ Supported by a grant from the Josiah Macy Jr. Foundation.

variables encountered in any such clinical study necessitate cautious interpretation. However, it may be said that, with certain unexplained exceptions, the level of phosphatase activity of the serum in the adult appears to be peculiarly sensitive to any significant compromise of the patency of the extra- or intrahepatic biliary system, but is relatively unaffected by even extensive liver parenchymal injury *per se*. The determination of serum phosphatase activity in this sense complements the so-called "liver function" tests, which afford a measure of injury to the liver parenchyma, and the dye retention tests, which are inapplicable in the presence of jaundice.

The absolute values reported in our tables and the empirical levels derived in our statistical analysis refer to the Bodansky method for the determination of serum phosphatase activity (4). In our hands, as in those of Bodansky, this method gives a range of 1 to 4 Bodansky units per 100 cc serum for normal adults (1). Serum bilirubin was estimated by the method of Thannhauser and Andersen (5). The results of various other determinations are not recorded, with a few exceptions that bear directly upon the interpretation of serum phosphatase values.

RESULTS IN CASES 124 TO 358²

1 Jaundice due to obstruction of the extrahepatic biliary tract

A. Pre-operative values in 27 proven cases of common duct stone (Table IA), 19 proven cases of carcinoma of the head of the pancreas or of the extrahepatic biliary tract (Table IB), and 8 proven cases of obstruction of miscellaneous origin (Table IC). The phosphatase activity of the serum was definitely increased in every one of these 54 cases of obstructive jaundice. Initial values in 49 cases ranged from 113.1 to 10.9 Bodansky units per 100 cc. serum. Values of less than 10 Bodansky units were obtained in 5 patients, of whom 3 were found to have calculi in the common duct with incomplete obstruction, 1 was a typical case of carcinoma of the head of the pancreas with complete obstruction of the common bile duct, and 1 was a curious case of

carcinoma arising from the proximal end of the common bile duct, with complete obstruction. The serum phosphatase values subsequently rose in this instance as the serum bilirubin level fell, for reasons that were never explained. The patient was not studied in the last months of life when jaundice recurred.

The total cholesterol content of these sera is recorded for comparison with the serum bilirubin and phosphatase levels. The significance of this comparison will be considered in the discussion.

B. Post-operative serum phosphatase values, with special reference to persisting external biliary fistulae. Serum phosphatase activity was determined post-operatively in 11 patients of the present series, at intervals varying from 3 days to 9 months after surgical intervention. Usually a roughly parallel trend in serum phosphatase and bilirubin was observed post-operatively. But sometimes there was a dissociation in serum phosphatase and serum bilirubin levels as illustrated particularly by the following 3 patients who developed persistent external biliary fistulae after cholecystectomy. It will be noted that, when such a dissociation in serum phosphatase and serum bilirubin levels occurred, the increased level of serum phosphatase activity paralleled the course of clinical complications, whereas the serum bilirubin showed little or no rise.

T. G., aged 45, developed bile peritonitis following cholecystectomy without drainage. Subsequent exploration disclosed disruption of the ligated cystic duct stump. The serum bilirubin rose to 5.2 mgm per cent, perhaps due to resorption of intraperitoneal bile, but the serum phosphatase level remained within essentially normal limits (4.7 Bodansky units) so long as bile drained freely into the abdominal cavity. After correction of the leak and establishment of temporary external biliary drainage, the jaundice eventually cleared, the biliary fistula gradually closed, the stools showed bile and the patient became afebrile. He returned later with chills, fever and malaise suggesting cholangitis, the symptoms subsiding after several weeks. Similar episodes recurred for the next 6 months. Throughout this period, he never developed jaundice, but the serum phosphatase activity was persistently elevated, ranging from 22.5 to 36.0 Bodansky units per 100 cc. As the febrile attacks gradually subsided, the serum phosphatase values began to fall.

A. P. (Case 6), following cholecystectomy, developed a stricture of the common bile duct with serum bilirubin of 10.7 mgm. per cent and serum phosphatase activity of 25.2 Bodansky units per 100 cc. Plastic repair of the common duct and establishment of a biliary fistula re-

² To avoid confusion with the 123 cases previously reported (1), the patients in the present series are numbered beginning with 124.

sulted in subsidence of jaundice but convalescence was stormy until, a month later a perihaptic abscess was drained. After several uneventful months jaundice re-occurred (serum bilirubin 7.2 mgm. per cent) with a serum phosphatase level of 349 Bodansky units per 100 cc. Exploration disclosed cicatrization of the repaired common bile duct. External drainage of bile again resulted in subsidence of jaundice but with cessation of drainage, fever chills and jaundice returned. For the next 2 years intermittent closure of the biliary fistula was associated with similar episodes suggesting cholangitis the serum bilirubin fluctuating between 8.8 and 3.0 mgm. per cent

the serum phosphatase level remaining in the region of 30 Bodansky units. Finally choledochoduodenostomy was performed (at which time biliary cirrhosis was noted) and this apparently effected adequate internal drainage of bile. The patient has been virtually asymptomatic for 1 year and the serum phosphatase has fallen to 18.3 Bodansky units per 100 cc. with a trace of icterus

The last patient R. R. aged 28 survived an even more complex sequence of post-cholecystectomy complications with an accompanying dislocation of serum phosphatase and bilirubin trends. In this instance, post-operative recurrence of severe jaundice suggested possible stricture

TABLE I

Summary of analyses of the blood in fifty-four cases of jaundice due to common bile duct obstruction
(Diagnosis established at operation or autopsy in each instance)

Number	Sex	Age years	Cause of obstruction	Approximate date of onset of jaundice	Bile in stool	Date	Serum			Remarks
							Phosphatase	Bilirubin	Cholesterol	
							Bodansky units per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	
A. CALCULI IN COMMON BILE DUCT										
124	♂	53	Choledocholithiasis, carcinoma of body of pancreas; free metastases	2 weeks	+	December 30, 1937	42.0	11.2	231	Operation January 6, 1938.
125	♀	53	Choledocholithiasis; sub-acute pancreatitis	1 week	±	February 26, 1939	37.1	2.4	270	Choledochostomy February 27 1939; pro-
126	♂	53	Choledocholithiasis; biliary cirrhosis?	6 months	+	April 15, 1939	27.3	2.3	Trace	operation biliary drainage.
					+	December 23, 1938	24.7	2.0	189	Cholecystectomy, choledochostomy Jan-
					+	January 24, 1939	19.8	2.2	211	20, 1939 free biliary drainage.
					+	July 15, 1939	17.2	0.0	240	
					+	July 24, 1939	14.8	0.0	218	
					+	August 8, 1939	15.0	2.8	140	
127	♀	53	Choledocholithiasis	1 day	+	May 23, 1939	24.2	2.0	200	Operation May 30, 1939.
128	♀	50	Choledocholithiasis, biliary cirrhosis	3 weeks	+	July 6, 1938	21.2	2.2	191	Cholecystectomy, choledochostomy July
					+	July 15, 1938	22.7	4.7	147	15, 1938; free biliary drainage. Trans-
					+	September 12, 1938	5.9	Trace	134	verse abdominal pain, skin, fever 2
					+	December 8, 1938	7.4	Trace	134	months after operation.
					+	April 13, 1939	4.0	0	133	
129	♀	45	Choledocholithiasis	1 week	0	October 10, 1938	20.9	10.0	100	Operation October 17 1938.
130	♀	45	Choledocholithiasis, carcinoma of head of pancreas, free metastases	16 months	0	March 8, 1937	19.9	15.5	227	Operation March 10, 1937.
131	♂	75	Choledocholithiasis	6 weeks	±	June 10, 1939	18.2	8.8	347	Operation June 24, 1939.
132	♂	45	Choledocholithiasis	1 week	+	January 20, 1938	18.7	12.8	250	Operation February 14, 1938.
133	♀	53	Choledocholithiasis	4 days	+	February 24, 1939	18.4	9.4	244	Cholecystectomy, choledochostomy Feb-
					+	March 13, 1939	6.8	Trace	288	ruary 27 1939 icteric course, subacute
					+	February 12, 1939	16.2	2.8	193	diagnosis.
134	♂	56	Choledocholithiasis, carcinoma of head of pancreas; biliary cirrhosis	6 weeks	0	February 12, 1938	18.7	4.3	340	Cholecystectomy March 24, 1938; Night
					+	March 17 1938	20.1	11.0	418	biliary drainage.
135	♂	37	Choledocholithiasis	2 weeks	+	July 20, 1938	19.0	2.0	282	Operation July 25, 1938.
136	♂	37	Choledocholithiasis	6 months	+	May 25, 1939	14.5	0.3	227	Operation June 3, 1939.
137	♀	52	Choledocholithiasis	4 days	+	June 15, 1939	16.3	1.9	175	Operation June 24, 1939.
138	♀	50	Choledocholithiasis	4 days	+	July 24, 1939	16.1	4.0	167	Cholecystectomy August 1, 1939; inter-
					+	August 8, 1939	8.8	6.0	119	mittent biliary drainage, spiking (fever
					+	August 10, 1939	11.8	6.7	115	cholangitis)
					+	January 25, 1938	12.5	8.8	350	Cholecystectomy choledochostomy Janu-
139	♀	53	Choledocholithiasis	2 weeks	0	February 11, 1938	7.0	10.3	88	ary 25, 1938, free bile drainage. Autop-
					+	February 11, 1938	12.5	8.8	350	opsy February 13, 1938, terminal hepatitis.
					+	February 11, 1938	7.0	10.3	88	Operation February 4, 1938 free biliary
					+	February 11, 1938	12.5	8.8	350	drainage.
140	♂	54	Stones in cystic duct and ampulla, intermittent common duct obstruction	2 days	+	February 8, 1938	13.2	2.0	115	Operation February 4, 1938 free biliary
141	♀	55	Choledocholithiasis	3 days	+	August 11, 1939	12.0	2.8	129	Operation August 16, 1939.
142	♀	51	Choledocholithiasis	5 months	+	April 21, 1937	12.8	4.7	174	Operation May 1, 1937.
143	♀	53	Choledocholithiasis	2 months	+	April 8, 1937	11.8	0.8	150	Operation April 12, 1937.
144	♂	50	Choledocholithiasis	2 weeks	0	January 24, 1938	11.0	8.3	286	Operation January 24, 1938.
145	♂	43	Choledocholithiasis	4 days	+	December 8, 1938	11.4	4.7	133	Operation December 12, 1938.
146	♀	23	Choledocholithiasis	4 days	±	April 25, 1939	11.3	10.7	324	Operation May 4, 1939.
147	♀	34	Choledocholithiasis	1 day	+	May 23, 1939	11.3	2.5	153	Operation May 30, 1939.
148	♂	84	Choledocholithiasis; suppurative pyelophlebitis	4 days	+	June 23, 1937	0.8	2.0	205	Autopsy June 24, 1937.
149	♀	39	Ball-valve stone in cystic duct, intermittent common duct obstruction	6 days	+	May 23, 1939	6.9	6.0	153	Operation June 2, 1937.
150	♂	38	Choledocholithiasis, pancreatitis	3 months	+	January 14, 1938	6.3	2.0	223	Operation January 9, 1938.

TABLE I—Continued

Number	Sex	Age	Cause of obstruction	Approximate dura- tion of jaundice be- fore initial blood analysis	Bile in stool	Date	Serum			Remarks
							Phosphatase	Bilirubin	Cholesterol	
		years					Bodansky units per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	
B CARCINOMA OF HEAD OF PANCREAS OR OF EXTRAHEPATIC BILIARY TRACT										
151	♀	38	Carcinoma of head of pancreas, liver metas- tases	5 months	0	April 9 1937	113.1	9.4		Cholecystogastrostomy April 19 1937
					0	April 13 1937	101.5	11.0	1,515	
152	♂	49	Carcinoma of head of pancreas	1 year	+	April 28, 1937	29.4	3.7	707	
153	♂	52	Carcinoma of head of pancreas? chronic pan- creatitis obstructing common duct?	9 weeks	0	July 18, 1938	63.8	15.0	417	Operation July 22, 1938.
154	♂	70	Carcinoma at junction of main hepatic ducts	7 weeks	0	April 15, 1939	48.8	11.5	347	Operation April 20, 1939
155	♂	65	Carcinoma of head of pancreas	4 weeks	0	June 28, 1939	41.5	15.8	387	Autopsy July 5, 1939
156	♀	67	Carcinoma at junction of main hepatic ducts	2 months	0	February 24, 1938	33.7	19.0	248	Cholecystojejunostomy March 3, 1938.
					+	March 8, 1938	18.5	13.6	290	
157	♂	64	Carcinoma of head of pancreas	1 week	0	June 15, 1937	27.6	15.0	675	
					+	December 7 1938	19.7	5.4	275	Autopsy June 30, 1937
					+	December 13, 1938	20.2	8.1	278	Operation December 23, 1938.
					+	December 16 1938	20.7	9.0		
158	♂	48	Carcinoma of head of pancreas	6 weeks	0	October 8, 1937	17.8	12.5	291	
159	♂	49	Carcinoma of head of pancreas, liver metas- tases	6 weeks	0	March 4, 1938	18.9	7.4	208	Operation October 15, 1937
										Operation March 11, 1938.
160	♀	72	Carcinoma of gall bladder or bile duct	2 weeks	+	July 13, 1939	16.2	17.6	308	Operation July 26, 1939
161	♂	70	Carcinoma of head of pancreas? chronic pan- creatitis obstructing common duct?	2 months	±	July 20 1939	19.2	18.0		Operation July 18, 1939
					±	July 10 1939	15.8	4.7	94	
162	♀	75	Carcinoma of head of pancreas	2 weeks	0	January 28, 1939	15.4	21.4		
					0	February 3, 1939	13.0	17.4		Cholechooduodenostomy February 4, 1939. Enlarged liver soon obviously metastatic.
					±	February 7, 1939	13.4	13.6	242	
163	♂	62	Carcinoma of head of pancreas	6 weeks	0	July 7 1938	13.8	18.8	525	
164	♀	62	Carcinoma of gall bladder, liver metastases	4 weeks	0	July 31, 1939	12.8	21.0	234	Operation July 14, 1938.
					0	August 5, 1939	13.7	18.8		Operation August 11, 1939
165	♂	48	Carcinoma of common bile duct	1 week	+	June 19, 1935	12.1	2.0	250	Autopsy August 22, 1935
166	♂	55	Carcinoma of ampulla of Vater	2 weeks	0	December 29, 1938	12.0	6.5	270	Operation March 6, 1939
					±	January 8, 1939	10.7	6.0		
					±	January 30, 1939	15.7	6.9		
					±	February 14, 1939	15.5	9.4	255	Operation October 6, 1937
					±	March 1, 1939	12.4	11.7		
167	♂	64	Carcinoma of head of pancreas	5 weeks	0	September 22 1937	10.9	12.1	625	
					±	September 27 1937	11.8	16.6	586	Cholecystojejunostomy December 30, 1938
168	♂	58	Carcinoma of body and head of pancreas	6 weeks	±	December 21, 1938	9.6	20.0	242	
					±	December 23, 1938	8.6	21.2		
					0	January 4, 1939	7.7	20.0	172	Exploration March 23, 1937
					0	March 9, 1937	8.7	20.6	288	
169	♀	60	Carcinoma of main hepatic ducts	10 weeks	0	March 17 1937	13.3	28.0		
						October 13 1937	28.2	2.0	191	Autopsy April 4, 1938.
						October 26, 1937	26.6	2.0		
C OBSTRUCTION OF COMMON BILE DUCT DUE TO MISCELLANEOUS CAUSES										
170	♂	61	Carcinoma of esophagus with extension to and occlusion of common duct	4 weeks	0	September 1, 1938	41.0	18.8		Autopsy September 13, 1938.
171	♀	29	Post-operative benign stricture of common bile duct	3 months	+	December 14 1937	36.4	3.2	340	Operation December 20, 1937
172	♀	50	Carcinoma, origin unknown obstruction of common bile duct	4 weeks	+	October 14, 1938	17.8	3.1		Operation October 22, 1938.
173	♂	4 mths.	Congenital stricture of bile ducts	4 months	0	August 3, 1939	17.1	9.0	167	Operation August 10, 1939
174	♀	32	Benign stricture of common bile duct	3 years	+	June 30, 1937	16.8	6.8		Operation August 16, 1937
					+	July 6, 1937	11.8	3.0		Operation August 11, 1939
175	♂	38	Carcinoma, origin unknown obstruction of common bile duct	3 weeks	±	July 27, 1939	15.5	4.8	223	
					±	August 5, 1939	16.2	9.2		
176	♂	60	Carcinoma of stomach, extension to and oc- clusion of common bile duct	5 weeks	+	February 21, 1938	14.5	10.8	278	Operation March 12, 1938
177	♀	65	Chronic pancreatitis, obstruction of common bile duct	1 month	+	May 3, 1939	10.9	1.5	197	Operation May 18, 1939
					+	May 17 1939	19.3	1.4		

of the common bile duct, although the serum phosphatase was only 7.8 Bodansky units with a serum bilirubin of 9.2 mgm. per cent. Exploration disclosed a patent common duct of normal caliber but free of bile. The operative findings suggested that the jaundice was of intra-hepatic origin, probably hepatitis. During this procedure, the duodenum was entered inadvertently and in the course of her stormy convalescence, a duodenal fistula

developed. Through this bile drained freely, with subsidence of jaundice and return of the serum phosphatase to 4.8 Bodansky units per 100 cc. The fistula closed eventually but transient episodes of chills, fever and malaise have recurred at frequent intervals since. Significant clinical jaundice has not developed, yet the serum phosphatase level rose and has remained at about 18 Bodansky units per 100 cc.

2 Jaundice due to hepatitis

Tables II and III together summarize our results in 67 cases of jaundice classified on clinical grounds as due to hepatitis. The distribution of serum phosphatase values in this group is not as

TABLE II

Summary of analyses of blood in forty-one cases of jaundice with a clinical course consistent with hepatitis of undetermined etiology (calarrhal jaundice)

Number	Sex	Age	Approximate duration of jaundice		Bile in stool	Date	Serum	
			Before initial blood analysis	Total duration			Phosphatase	BIL-ruhin
		years	days	weeks				
178	♂	45	20	5	+	February 7, 1939 February 8, 1939 February 11, 1939 March 5, 1939	15.3 13.1 11.8 8.9	7.3 0.7 5.0 Trace
179	♀	29	2	2	+	April 15, 1939 April 19, 1939 April 24, 1939 May 2, 1939	13.2 10.3 7.5 5.0	8.0 4.8 L.O Trace
180	♀	13	7	1	+	November 17, 1938	12.1	10.0
181	♀	51	31	10	0	June 30, 1938 July 8, 1938 July 15, 1938	11.1 9.2 8.9	23.0 10.0 8.9
182*	♂	48	28	13	+	July 7, 1939 July 17, 1939 July 21, 1939	10.5 9.5 9.1	8.7 8.4 17.5
183	♂	35	5	2.5	+	January 27, 1939 February 2, 1939	9.7 7.7	6.3 7.3
184	♂	30	14	3	+	June 10, 1937 June 19, 1937	9.8 8.5	6.0 2.7
185	♂	42	0	3.5	+	December 24, 1938 January 11, 1939	8.3 5.9	8.9 2.7
186	♂	49	9	3	+	August 19, 1939	9.0	24.3
187	♂	23	23	6.5	+	January 6, 1939 January 28, 1939 February 14, 1939	8.3 7.5 4.4	12.1 7.5 3.5
188	♀	49	9	2	+	May 2, 1937 May 11, 1937 May 14, 1937	8.8 6.9 6.0	8.3 8.4 2.7
189	♀	74	25	6	+	November 6, 1938	8.4	10.3
190	♂	29	4	2	0	February 27, 1939 March 6, 1939 April 2, 1939	7.9 7.1 2.5	4.9 8.0 Trace
191	♂	34	10	3	0	July 15, 1937	7.9	8.0
192	♀	23	4	1.5	+	February 15, 1939 February 20, 1939 June 20, 1939	7.8 8.5 3.3	4.8 8.3 9
193	♂	33	43	11	+	April 25, 1939 April 28, 1939	7.7 7.3	11.5 11.7
194	♀	23	7	3	+	September 21, 1938	7.7	10.7
195	♀	67	20	12	0	January 6, 1939 January 10, 1939	7.5	12.5 6.0
196	♂	48	18	9	+	June 6, 1939 June 13, 1939 July 6, 1939	7.4 7.8 2.9	12.3 18.0 2.3

TABLE II—Continued

Number	Sex	Age	Approximate duration of jaundice		Bile in stool	Date	Serum	
			Before initial blood analysis	Total duration			Phosphatase	BIL-ruhin
197	♂	23	3	1	+	October 15, 1938	7.4	2.9
198	♀	43	7	8	+	December 3, 1937 December 15, 1937	7.2 5.4	11.7 10.1
199	♂	22	3	2	+	March 14, 1938 March 16, 1938 March 22, 1938	7.2 6.5 8.0	5.4 3.1 8.0
200	♂	34	4	1.5	+	October 2, 1937 October 8, 1937	7.2 7.3	6.1 7.5
201	♀	15	20	11	+	February 25, 1939 February 25, 1939 March 30, 1939 April 3, 1939	6.9 6.9 7.3 7.4	10.4 8.8 8.3 8.0
202	♀	49	78	18	+	October 12, 1937 October 21, 1937 November 14, 1937 January 4, 1938	6.8 4.3 2.1 2.5	5.0 2.3 2.0 Trace
203	♀	20	1	1.5	+	February 11, 1938	6.7	3.8
204	♀	29	8	2.5	+	October 20, 1938	6.5	9.6
205	♀	23	8	3	+	August 4, 1938 August 15, 1938	6.4 4.7	7.8 2.1
206	♂	37	9	3.5	0	November 25, 1938 December 1, 1938	6.1 4.5	4.5 2.0
207	♂	31	23	7	0	March 2, 1939	5.8	18.0
208	♀	34	8	3	+	July 1, 1939	5.6	9.4
209	♂	39	18	3.5+	+	October 25, 1937	5.6	4.4
210	♂	23	21	4	+	July 21, 1938 July 25, 1938	5.5 5.3	18.0 3.9
211	♂	34	8	2	+	August 9, 1938	5.4	9.4
212	♂	42	14	5+	+	August 12, 1938 August 22, 1938 August 30, 1938	5.1 4.7 4.3	21.0 18.0 12.3
213	♂	23	3	1.5	0	June 30, 1938	5.1	7.5
214	♀	34	3	2	+	January 12, 1938	4.8	4.3
215	♂	28	3	2	+	January 5, 1939	4.6	6.8
216	♂	32	23	13	+	October 26, 1937 November 14, 1937 December 14, 1937	4.3 3.9 3.3	18.8 8.0 2.5
217	♀	37	4	1.5	+	March 10, 1938	3.1	6.9
218†	♂	10	18	2.5	+	February 25, 1937	2.8	8.4

† Subsequently found to have hemolytic jaundice, acquired.

sharply defined in relation to the empirical level of 10 Bodansky units as in the cases of obstructive jaundice the phosphatase levels did not reach 10 Bodansky units per 100 cc. serum in 49 instances, whereas 18 cases exceeded this figure. However the results show greater consistency if those patients whose jaundice followed upon the

* Stone subsequently found in common duct.

TABLE III

Summary of analyses of the blood in twenty-six cases of jaundice following exposure to known hepatotoxic agents

Number	Sex	Age	Cause of jaundice	Approximate duration of jaundice		Urobilin in stool	Date	Serum	
				Before initial blood analysis	Total duration			Phosphatase	Bilirubin
		years		days	weeks			Bodansky units per 100 cc.	mgm. per 100 cc.
219	♀	53	Arsenical therapy	7	> 7	± ± 0 0 +	May 31, 1939 June 5, 1939 June 15, 1939 June 22, 1939 June 29, 1939	19.5 30.9 41.6 31.5 25.0	5.3 9.9 16.6 13.8 12.9
220	♂	29	Arsenical therapy	20	8	0 ± + +	May 20, 1938 June 1, 1938 June 14, 1938 June 21, 1938	17.6 13.8 11.6 11.8	17.0 15.0 12.5 9.4
221	♂	35	Arsenical therapy	70+	14+	+ + +	July 24, 1939 July 28, 1939 August 18, 1939	16.7 14.9 24.7	6.5 6.5 4.7
222	♀	22	Cinchophen	20	8	+ +	April 21, 1939 May 1, 1939	15.8 13.8	4.0 2.0
223	♀	25	Arsenical therapy	7	> 3	+	June 8, 1939	14.5	17.0
224	♀	57	Arsenical therapy	7	8	0 + + +	January 19, 1937 January 26, 1937 February 2, 1937 March 4, 1937	14.0 7.8 11.2 6.8	14.2 15.0 12.5 2.0
225	♀	36	Arsenical therapy	60±	25	0 ± + +	July 26, 1938 September 1, 1938 September 13, 1938 September 27, 1938	12.7 18.5 20.1 13.9	10.4 15.0 11.7 9.4
226	♂	19	Arsenical therapy	7	4	+ +	March 9, 1938 March 22, 1938	12.2 14.3	7.0 2.2
227	♂	53	Arsenical therapy	9	20	+ +	March 17, 1938 March 21, 1938	12.1 9.0	13.2 7.4
228	♂	33	Sulfanilamide	2	1		April 8, 1937 July 10, 1939 July 16, 1939	11.3 11.4 9.1	1.0 Trace
229	♂	24	Arsenical therapy	6	2+	+ +	March 27, 1939 April 3, 1939	10.8 20.4	7.9 11.5
230	♂	30	Sulfanilamide	5	5	+	May 24, 1937 June 1, 1937 June 7, 1937 June 16, 1937 June 28, 1937	10.4 11.9 10.5 10.9 6.1	2.0 2.0 7.1 2.0 1.0
231	♂	43	Arsenical therapy	63	13	+	July 29, 1939 August 7, 1939	10.0 9.0	16.0 16.5
232	♀	57	Phosphorus*	2	4	?	August 11, 1938	8.7	2.7
233	♂	26	Arsenical therapy	?	?	+	February 3, 1937 March 9, 1937	8.4 3.2	8.3 Trace
234	♂	46	Arsenical therapy	14	4	+ +	June 14, 1937 June 24, 1937	8.2 6.2	4.2 3.0

TABLE III—Continued

Number	Sex	Age	Cause of jaundice	Approximate duration of jaundice		Urobilin in stool	Date	Serum	
				Before initial blood analysis	Total duration			Phosphatase	Bili rubin
		years		days	weeks			Bodansky units per 100 cc.	mg. per 100 cc.
235	♀	38	Sulfapyridine	1	5	+	January 24 1939	7.9	6.5
							January 27 1939	13.6	2.0
							February 1 1939	8.4	Trace
236	♂	37	Arsenical therapy	15	4+	+	January 14 1937	7.5	15.0
							January 18 1937	4.6	14.4
							January 21 1939	4.1	11.2
237	♀	35	Arsenical therapy	35	12	+	June 3 1938	6.9	13.0
						+	June 13 1938	8.7	9.4
						+	June 27 1938	8.9	5.0
						+	July 5 1938	8.6	4.3
238	♂	34	Arsenical therapy	16	4	+	February 5 1937	6.5	11.4
239	♂	71	Carbon tetrachloride*	7	1	+	March 22 1937	6.3	14.0
240	♂	22	Arsenical therapy	60±	10	0	August 26 1936	6.0	8.3
241	♀	32	Sulfapyridine	1	< 1	+	December 27, 1938	4.9	3.8
							January 5 1939	3.3	1.5
242	♀	37	Carbon tetrachloride	?	2±	+	May 22 1937	4.7	4.5
							May 29 1937	4.4	3.5
							June 2 1937	5.0	4.0
							June 8 1937	4.6	2.0
243	♀	47	Sulfanilamide	?	1	+	December 24 1937	4.1	Trace
						+	January 3 1938	7.6	2.0
244	♂	42	Sulfanilamide	1	3	+	April 9 1937	3.1	3.0

* Fatal termination.

administration of hepatotoxic drugs are segregated from the miscellany of cases which it is convenient clinically to group together as "hepatitis". This division for which there is other justification ordinarily can be made without difficulty by reference to the case history.

Table II includes 41 patients with jaundice of indeterminate etiology in whom the clinical course with few exceptions, was typical of so-called "catarrhal" jaundice. The phosphatase activity of the serum in all but 5 of these patients was less than 10 Bodansky units per 100 cc. The 5 exceptions include one 13 year-old patient whose serum phosphatase level of 13.1 Bodansky units was little, if at all above the normal maximum for that age period. In many of the cases in this group with comparatively little rise in serum phosphatase activity the degree of jaundice and the clinical course were indicative of severe paren-

chymal involvement. In some, acholic stools were present for variable, occasionally protracted periods.

Table III includes 26 patients with jaundice following exposure to known hepatotoxic agents for the most part luetics after intravenous administration of arsphenamine. In some of these, icterus developed rapidly early in the course of treatment, the highest values in Table III falling in this group. In others, icterus appeared as late as 6 months following cessation of treatment.

The dispersion in the cases comprising Table III is striking no less than 14 cases (about half) presented serum phosphatase values greater than 10 Bodansky units at some time in the course of their jaundice in one instance reaching a level of 41.6 Bodansky units per 100 cc. serum. There is some evidence that the dispersion in serum phosphatase values observed in arsphenamine

jaundice may have clinical significance since many patients with markedly increased serum phosphatase activity also presented other peculiarities (clinical as well as in laboratory data) suggesting an obstructive rather than hepatogenous type of jaundice. For example, Hanger (6) pointed out that the sera of patients with arsphenamine jaundice and markedly increased serum phosphatase activity fail to flocculate cephalin-cholesterol emulsions, a sensitive test for hepatitis. Moreover, the serum cholesterol values in this group rose, in contrast to the usually normal or lowered levels in hepatogenous jaundice, the rise occurring after icterus had been present for some time and tending to persist after jaundice had subsided. Clinically, this group was characterized (6) by acute onset in the form of a typical delayed reaction to

the second or third arsphenamine injection, by intense icterus and pruritus, acholic stools for long periods, and relative freedom from gastrointestinal upsets.

The cases in Table III with little rise in serum phosphatase activity, on the other hand, for the most part presented a clinical and laboratory picture more in keeping with that of hepatogenous jaundice.

3 Hemolytic jaundice

Table IV summarizes the results in 12 of our patients with hemolytic jaundice of diverse etiology. Case 245 was an infant who was deeply icteric at birth, the hyperbilirubinemia gradually subsiding within 6 weeks, an instance, apparently, of unusually severe and protracted "physiologi-

TABLE IV
Summary of analyses of the blood in twelve cases of hemolytic jaundice

Number	Sex	Age	Date	Serum		Diagnosis
				Phos- phatase	Bilirubin	
		years		<i>Bodansky units per 100 cc.</i>	<i>mgm per 100 cc</i>	
245	♂	2 weeks	January 22, 1937 February 2, 1937	13.7	15.0 (direct reaction) 7.4	Unusually marked "physiological jaundice" of newborn with protracted but benign course
246	♂	28	December 19, 1935 December 23, 1935 December 27, 1935 February 3, 1936 May 12, 1937 December 11, 1937 September 17, 1938 September 23, 1938	13.6 9.9 5.8 3.5 3.9 3.8 3.5	18.9 (direct reaction) 10.0 (direct reaction) 9.4 (direct reaction) 5.4 (biphasic reaction) 6.1 (indirect reaction) 3.4 25.0 (direct reaction) 4.0 (direct reaction)	Sickle cell anemia with related bone changes, hemolytic crises Hemolytic crisis
247	♂	57	May 1, 1939	3.9	5.4 (biphasic reaction)	Familial (spherocytic) hemolytic jaundice
248	♀	36	June 20, 1938	3.8	2.9 (indirect reaction)	Familial (spherocytic) hemolytic jaundice
249	♂	18	May 5, 1937	2.6	4.7 (indirect reaction)	Acquired hemolytic jaundice, cause unknown, splenomegaly
250	♀	58	September 22, 1938	2.6	2.0 (indirect reaction)	Pernicious anemia
251	♂	57	May 13, 1938	2.5	3.0 (indirect reaction)	Pernicious anemia
252	♀	70	June 1, 1936	2.3	2.0 (indirect reaction)	Pernicious anemia
253	♂	62	June 15, 1937	2.3	3.4 (indirect reaction)	Pernicious anemia
254	♂	35	July 20, 1938	2.0	3.0 (indirect reaction)	Familial (spherocytic) hemolytic jaundice
255	♂	74	May 27, 1938	2.0	2.0 (indirect reaction)	Pernicious anemia
256	♀	61	March 19, 1937	1.9	2.0 (indirect reaction)	Pernicious anemia

cal" jaundice. The value 13.7 Bodansky units per 100 cc. is little, if at all, above the normal maximum for that age period. Case 246 was a colored male with severe sickle cell anemia and related skeletal changes who experienced several episodes of recurring jaundice. The first of these, in 1935, was an afebrile attack of deep jaundice which subsided spontaneously after 2 weeks. Marked enlargement and tenderness of the liver, choluria, normal stools and negative x rays for opaque biliary calculi, characterized this episode, the precise nature of which remained uncertain. In 1938 severe jaundice recurred with a sharp drop in the erythrocyte count typical of 'hemolytic crisis'. Because of painful attacks suggesting biliary colic and x ray evidence of gall bladder disease, the possibility of obstruction due to pigment stones was suggested. The serum phosphatase level was within normal limits despite very marked hyperbilirubinemia. Exploration revealed calcium bilirubinate 'mud' in the gall bladder but no obstruction of the common bile duct, which was not dilated and through which saline could be irrigated freely.

As indicated in Table IV, the phosphatase activity of the serum is consistently within normal limits in hemolytic jaundice. There is no difficulty in establishing the diagnosis of hemolytic jaundice on other grounds, however, and we regard the use of the method in this type of jaundice as of chiefly theoretical interest, though in occasional obscure instances such as Case 246 the determination may be of distinct aid.

4 Cirrhosis of the liver

Table V summarizes our results in 30 patients with cirrhosis of the liver in whom the diagnosis could be established at necropsy or by liver biopsy. This is a heterogeneous group. The majority of subjects were chronic alcoholics and at necropsy many were found to have a fatty type of Laennec's cirrhosis specified as alcoholic cirrhosis (7), when fatty degeneration was not striking but fibrosis was the chief abnormality the findings are designated more generally as Laennec's cirrhosis. The presence of extreme atrophy is recorded. The series further comprises 3 cases of "cardiac" cirrhosis in patients with long standing cardiac failure, 3 cases of hemochromatosis, 2 cases of

"toxic" cirrhosis associated with hyperthyroidism and 1 case of schistosomiasis of the liver with cirrhotic changes. Cases 257 and 263 were relatively young persons. In the former the clinical course was that of sub-acute yellow liver atrophy though the pathological findings were not so classified. Our proven instances of biliary cirrhosis were all patients with chronic obstructive jaundice and are included in Table I.

These patients for the most part presented the picture of hepatic cirrhosis in its more advanced stages. We have recorded the results of serum protein analyses (Howe's method was used) to afford another criterion of the degree of hepatic pathology. The highest value for serum phosphatase activity observed in this group was 20.3 Bodansky units per 100 cc. In only 6 patients were serum phosphatase levels of 10 Bodansky units or over obtained and 3 of these inconstantly exceeded that figure. Almost half of this group of advanced cirrhotics were found on at least one occasion to have essentially normal serum phosphatase levels. The results indicate that even advanced hepatic cirrhosis of the type included in Table V usually effects little rise in serum phosphatase activity. In biliary cirrhosis the values are quite consistently elevated.

5 Neoplastic involvement of the liver

Four proven cases of primary carcinoma of the liver (Table VIA), 10 cases of malignancy proven not to have liver involvement (Table VIB) and 22 cases of malignancy with proven metastases to the liver (Table VIC). In 2 of the cases of primary carcinoma of the liver, the tumor had its origin in the intrahepatic biliary system (cholangioma). 2 arose from hepatic cells (hepatoma) in association with advanced Laennec's cirrhosis. Not included in Table VI are 2 cases of carcinoma arising from the junction of the main hepatic ducts, which are recorded in Table IB (Cases 154 and 156) as instances of obstructive jaundice due to carcinoma at the origin of the common bile duct. Being in effect, if not literally instances of extrahepatic duct obstruction. The serum phosphatase activity of the cases comprising Table VIA was distinctly elevated, the values ranging from 33.1 to 11.5 Bodansky units per 100 cc. In 2 previously recorded instances of

primary carcinoma of the liver (Cases 99 and 118), the serum phosphatase levels were somewhat lower but still above those of most cases of uncomplicated hepatic cirrhosis. It would appear that the serum phosphatase activity tends to be higher in patients with primary carcinoma of the liver than in uncomplicated Laënnec's cirrhosis, with which the condition is most frequently confused clinically.

In considering patients with malignancy other than primary carcinoma of the liver, it is apparent from our data that the serum phosphatase level

depends largely upon the presence or absence of metastases to the liver or skeleton. In Table VIB are recorded serum phosphatase values obtained in 10 patients with neoplasia shown at necropsy not to have involved the liver. Cases 291 and 292, however, had extensive osteoplastic metastases to bone and are included to illustrate the marked increase in serum phosphatase activity usually accompanying this type of metastatic spread. Cases 293 and 294 exhibited a significant rise in serum phosphatase activity although no metastases were noted in the liver or bones at

TABLE V

Summary of analyses of the blood in thirty proven cases of cirrhosis of the liver

Number	Sex	Age	Basis for diagnosis	Duration of jaundice at time of blood analysis	Bile in stool	Date	Serum						Remarks
							Phosphatase	Bilirubin	Total protein	Albumin	Globulin	Euglobulin	
		years					Bodansky units per 100 cc.	mgm. per cent	per cent	per cent	per cent	per cent	
257	♀	21	Autopsy	5 months	0 0 0 ± ± ±	April 28, 1938 May 13, 1938 May 25, 1938 June 17, 1938 July 5, 1938 July 26, 1938	15.4 13.9 11.4 18.7 14.5 11.3	17.3 16.0 15.0 15.0 13.4 15.0	9.6 9.7 8.5 7.6 8.0 7.8	2.5 2.6 2.4 2.6 2.6 2.6	7.1 7.1 6.1 5.0 5.4 5.2	3.6 3.6 2.9 2.0 2.0 1.3	Diffuse atrophic Laënnec's cirrhosis, ascites, cholemia.
258	♀	35	Liver biopsy	Not known	++ ++ ++ ++ +	May 26, 1937 December 17, 1937 May 3, 1938 November 30, 1938 May 16, 1939	14.7 20.0 20.3 15.5 14.1	3.2 2.5 2.0 2.5 3.0	7.3 8.1 7.2 7.2 7.7	2.8 3.6 2.8 3.2 3.6	4.5 4.5 4.4 4.0 4.3	1.2 1.4 1.6 0.9 0.8	Chronic alcoholism. Laënnec's cirrhosis, diffuse, with hepatosplenomegaly.
259	♀	43	Autopsy	Not jaundiced	++ ++ +	April 28, 1937 May 20, 1937 June 14, 1937	10.6 10.9 4.4	0 0 0	5.0 5.2 5.1	2.6 3.0 2.1	2.4 2.2 3.0	0.4 0.6	Diffuse "cardiac" cirrhosis, adherent pericardium.
260	♂	32	Autopsy	Not jaundiced	+	November 18, 1936	10.1	1.0	4.2	2.6	1.6	0.3	Post-operative gastro-jejunal-colic fistula, malnutrition. Fatty liver, diffuse Laënnec's cirrhosis.
261	♀	43	Autopsy	Not jaundiced	++ +	April 20, 1938 May 16, 1938	9.2 4.7	1.0 0	6.7 7.1	3.6 3.6	3.1 3.5	0.3 0.5	Chronic alcoholism. Diffuse alcoholic cirrhosis. No marked atrophy.
262	♀	52	Autopsy	1 week	+	August 11, 1938	8.5	9.5	6.3	3.1	3.2	0.6	Chronic alcoholism. Diffuse cirrhosis, unclassified type, superimposed hepatitis?
263	♀	11	Liver biopsy	1 month	±	December 2, 1936 December 22, 1936	8.4 8.7	4.2	7.3	2.4	4.9		Diffuse Laënnec's cirrhosis with atrophy.
264	♀	45	Autopsy	Not jaundiced	+	November 22, 1937 December 1, 1937	8.2 7.4	0 trace	8.5 6.8	5.9 3.2	2.6 3.6		Marked "cardiac" cirrhosis, hepatomegaly. Rheumatic cardiac disease.
265	♂	64	Autopsy	Not known	+	May 31, 1938	8.1	2.2	5.9	2.1	3.3	0.9	Chronic alcoholism. Atrophic Laënnec's cirrhosis. Ascites.
266	♂	60	Autopsy	Not jaundiced	+	January 13, 1937	7.7	2.0	6.1	1.5	4.6	1.6	Chronic alcoholism. Atrophic Laënnec's cirrhosis, ascites.
267	♀	59	Autopsy	Not known	++ +	January 12, 1937 January 20, 1937	7.3 6.6	6.5 9.6					Chronic alcoholism. Diffuse alcoholic cirrhosis, ascites.
268	♀	37	Autopsy	1 week	++ +	March 24, 1939 March 31, 1939	6.4 6.2	2.0 3.4	6.2 6.2	3.5 3.2	2.7 3.0	0.6 0.6	Hyperthyroidism with patchy cirrhosis.
269	♀	65	Autopsy	Not jaundiced	+	January 27, 1939	6.2	trace	6.9	4.0	2.9	0.6	Hyperthyroidism with diffuse cirrhosis.
270	♂	29	Liver biopsy	Not jaundiced	++ +	February 15, 1939 March 14, 1939	5.9 10.0	0 trace	5.7 7.2	3.3 3.4	2.4 3.8		Diffusely nodular liver, no atrophy. Moderately advanced Laënnec's cirrhosis.
271	♂	48	Autopsy	3 months	++ ++ ++ +	October 28, 1938 November 28, 1938 December 15, 1938 January 25, 1939	5.8 10.1 7.4 10.7	2.0 3.3 3.8 3.0	6.1 5.9 6.3 6.2	1.3 1.3 1.7 2.0	4.3 4.1 4.6 4.2	1.2 1.1 1.3 1.1	Diffuse Laënnec's cirrhosis with atrophy. Ascites.

TABLE V—Continued

Number	Sex	Age	Basis for diagnosis	Duration of jaundice at time of blood analysis	Bile in stool	Date	Serum						Remarks
							Phosphatase	Bilirubin	Total protein	Albumin	Globulin	Ex-globulin	
		years					Bodansky units per 100 cc.	mgm. per cent	per cent	per cent	per cent	per cent	
272	♀	42	Autopsy	Not jaundiced	+	December 31, 1935 January 11, 1937 January 20, 1937 January 27, 1937 December 20, 1937 February 25, 1938	4.7 5.7 6.1 5.4 2.0 2.5	trace 2.0 trace trace 2.0 trace	8.3 8.3 8.3 8.7 8.5 6.5	2.3 2.3 2.3 2.4 2.1 2.0	4.1 4.3 4.0 4.3 4.4 4.5	1.3 1.7 2.3 2.7 2.3 2.4	Atrophic Laënnec's cirrhosis, diffuse. Ascites.
273	♂	47	Autopsy	Not jaundiced	+	March 16, 1937	4.7	0	7.0	3.9	3.1	0.4	Diffuse "cardiac" cirrhosis; coronary sclerosis, hepatomegaly (congestive).
274	♂	57	Autopsy	3 weeks	0	December 11, 1937	4.7	18.0	6.3	2.3	3.9	1.4	Diffuse Laënnec's cirrhosis with atrophy; acute cholangitis, ascites.
275	♂	60	Autopsy	3 weeks	0	February 27, 1938 March 4, 1938	4.4 2.8	24.3 27.0	6.7 7.3	2.3 2.5	4.4 4.7	1.5	Chronic alcoholism. Atrophic Laënnec's cirrhosis, diffuse. Ascites, cholecystitis.
276	♂	64	Skin biopsy	Not jaundiced	+	June 7, 1937	4.2	0	7.3	4.3	3.0	0.4	Hemochromatosis. Hepatomegaly, diabetes.
277	♀	40	Autopsy	Not jaundiced	+	September 24, 1937	2.9	0	6.9	2.7	4.3	1.4	Diffuse Laënnec's cirrhosis, early. Leuc.
278	♀	54	Autopsy	1 week	±	February 24, 1936	4.2	29.0	6.5	2.5	4.3	0.7	Chronic alcoholism, diffuse alcoholic cirrhosis; ascites.
279	♂	53	Cholecystectomy	Not jaundiced	+	January 25, 1937	2.5	trace	5.5	3.3	3.3	0.7	Chronic alcoholism. Diffuse cirrhosis.
280	♀	68	Autopsy	Not jaundiced	+	January 18, 1937	3.4	trace	6.9	4.1	2.8	0.8	Coarse lobular cirrhosis, leuc.
281	♂	64	Autopsy	Not jaundiced	+	January 14, 1936	3.3	2.0	6.5	3.6	2.9		Hemochromatosis. Diffuse cirrhosis with hepatomegaly.
282	♂	59	Autopsy	Not known	+	June 8, 1937	3.3	6.5	7.1	2.6	3.5	1.3	Chronic alcoholism. Diffuse Laënnec's cirrhosis, no atrophy.
283	♂	74	Autopsy	Not jaundiced	+	March 22, 1939	3.1	1.5	7.1	4.0	3.1	0.3	Diffuse Laënnec's cirrhosis, moderately advanced.
284	♂	47	Autopsy	Not jaundiced	+	September 20, 1937 December 22, 1937	2.7 2.5	2.0 1.0	6.3 6.3	2.7 4.3	2.6 2.1	0.1	Hemochromatosis. Hepatomegaly, marked cirrhosis, diabetes.
285	♀	31	Liver biopsy	Not known	+	October 2, 1936	2.5	2.1					Bilestomatosis; diffuse cirrhosis.
286	♀	24	Liver biopsy	Not known	+	December 12, 1935	1.5	2.3	5.5	3.4	2.1		Diffuse Laënnec's cirrhosis, moderately advanced.

necropsy This result is contrary to our usual experience as indicated by the essentially normal values of the remaining cases in Table VIB

Table VIC comprises 22 cases of neoplasm with proven metastatic involvement of the liver, exclusive of all subjects in whom the primary tumor originated from or by extension impinged upon the extrahepatic biliary tract. Excluded also are all patients with bone metastases demonstrable by x ray or at necropsy with the exception of Case 320. It is assumed that with these restrictions a distinct increase in serum phosphatase activity may be attributed to the presence of secondary metastases to the liver

The serum phosphatase level was found to be variable in this group ranging from 23.9 to 4.0 Bodansky units per 100 cc. The values in 17

cases exceeded the maximum we usually find in non metastasizing malignancy (Table VIB). There were 5 instances, however, with serum phosphatase activity less than 5 Bodansky units despite definite metastatic involvement of the liver, and Case 320 presented both hepatic and skeletal (osteolytic) metastases without any significant rise in serum phosphatase activity (an exceptional occurrence in our experience). The patients with little or no elevation in serum phosphatase level were not icteric.

In general, the serum phosphatase activity tends to be higher in patients with diffuse spread of large nodules throughout the liver. Our highest values were in patients who showed jaundice, though some were only slightly icteric (Cases 301, 302), and in several instances the serum phos-

TABLE VI

Summary of analyses of the blood in four proven cases of primary carcinoma of the liver, ten cases of malignancy without liver metastases, and twenty-two cases of malignancy with proven involvement of the liver

Num ber	Sex	Age	Primary tumor, basis for diagnosis	Serum		Remarks
				Phos phatase	Bill rubin	
		years		Bodansky units per 100 cc	mgm per 100 cc	
A PRIMARY CARCINOMA OF THE LIVER						
287	♀	41	Cholangioma (autopsy)	25.8	4.7	April 20, 1938
				33.1	4.4	May 9, 1938
				29.7	3.2	June 1, 1938
288	♂	65	Hepatoma, cirrhosis (liver biopsy)	16.8	4.0	January 6, 1938
				27.4	7.5	January 24, 1938
289	♂	38	Hepatoma, cirrhosis (liver biopsy)	16.3	3.0	June 1, 1939
290	♀	57	Cholangioma (autopsy)	11.5	2.5	June 29, 1937
B MALIGNANCY WITHOUT LIVER METASTASES						
291	♂	67	Carcinoma of prostate gland (autopsy)	44.4	0	Extensive osteoplastic metastases to bone
292	♂	61	Carcinoma of prostate gland (autopsy)	21.6	0	Extensive osteoplastic metastases to bone
293	♀	43	Melanosarcoma (autopsy)	7.8	trace	Brain metastases, focal necroses of liver
				3.9	0	
294	♂	57	Carcinoma of colon (autopsy)	7.4	0	Large local abscess formation
295	♂	71	Carcinoma of stomach (autopsy)	4.9	1.0	Local metastases to nodes
296	♀	60	Carcinoma of lung (autopsy)	4.5	0	Metastases to lung, lymph nodes
297	♂	55	Oat cell tumor of lung (autopsy)	4.1	0	Metastases to brain, adrenals, etc
298	♀	47	Lymphosarcoma (autopsy)	3.9	0	Metastases to spleen, pelvis
299	♀	70	Carcinoma of colon (autopsy)	3.4	0	No metastases
300	♀	70	Lymphosarcoma (autopsy)	3.2	0	Metastases to lung, skin, spleen, etc
C MALIGNANCY WITH LIVER METASTASES						
301	♀	43	Primary not known (liver biopsy)	23.9	2.5	Many liver metastases, jaundiced 1 year
302	♀	39	Carcinoma of esophagus (autopsy)	21.5	2.7	Many liver metastases
303	♂	32	Reticulum cell sarcoma (autopsy)	20.7	5.0	Many liver metastases
304	♀	63	Carcinoma of colon (liver biopsy)	19.8	9.6	Many liver metastases, jaundiced 1 month
305	♀	36	Carcinoma of stomach (autopsy)	18.8	trace	Many liver metastases
306	♀	82	Carcinoma of tail of pancreas (autopsy)	18.3	0	Many liver metastases
307	♂	74	Primary not known (autopsy)	13.1	7.4	Many liver metastases, duration of jaundice not known
308	♂	47	Primary not known (celiotomy)	12.3	0.5	Many liver metastases
309	♂	60	Carcinoma of bronchus (autopsy)	10.4	2.7	Many liver metastases
310	♂	60	Sarcoma ? of testis (autopsy)	9.7	trace	Diffuse microscopic infiltration of liver, no gross nodules
311	♀	52	Melanosarcoma (liver biopsy)	9.0	8.3	Many liver metastases Duration of jaundice not known
312	♂	63	Carcinoma of kidney (autopsy)	8.5	0	Many liver metastases
313	♂	69	Carcinoma of prostate gland (autopsy)	8.4	0	Many liver metastases (Early bone invasion, osteolytic)
314	♀	51	Carcinoma of cervix (autopsy)	7.5	0	Many liver metastases
315	♀	40	Krukenberg tumor (autopsy)	6.7	trace	Many liver metastases, extensive osteolytic bone metastases
316	♀	35	Carcinoma of rectum (liver biopsy)	6.5	trace	Many liver metastases
317	♂	54	Carcinoma of stomach (autopsy)	6.3	0	Slight infiltration of liver
318	♂	62	Primary not known (liver biopsy)	4.9	trace	Many liver metastases
319	♀	63	Carcinoma of colon (autopsy)	4.4	0	Many liver metastases
320	♂	50	Oat cell tumor of lung (autopsy)	4.2	0	Few liver metastases, extensive osteolytic bone infiltration
321	♂	56	Hodgkin's sarcoma (autopsy)	4.1	0	Several liver metastases
322	♂	63	Carcinoma of colon (autopsy)	4.0	trace	One nodule in liver

phatase level was distinctly increased before jaundice developed (Cases 305, 306, 308). It has been our experience that significantly increased serum phosphatase activity occurs more consistently in this group of patients than does hyperbilirubinemia. Some of the most striking examples of this are not included in Table VI because, though the diagnosis of malignancy with liver metastases was obvious clinically permission for autopsy and proof of the diagnosis could not be obtained.

6 *Liver abscess chronic passive congestion of the liver, miscellaneous disorders of the liver, cases of gall bladder disease with indeterminate effect upon the patency of the common bile duct*

Table VIIA includes 9 cases of liver abscess of diverse etiology. The diagnosis was estab-

lished by exploration or necropsy except in 2 patients with amebic abscess in whom the diagnosis would seem beyond reasonable doubt on clinical and laboratory grounds. The serum phosphatase activity was variable but definitely increased in every instance ranging from 7.9 to 49.4 Bodansky units per 100 cc. The highest serum phosphatase levels occurred in jaundiced patients.

Table VIIB includes 10 patients with marked chronic passive congestion of the liver in decompensated cardiacs of long standing who at necropsy were found to have little or no "cardiac" cirrhosis. Congestive failure may cause some increase in serum phosphatase activity particularly in patients who develop clinically demonstrable jaundice, 2 such patients in our series (Cases 332 and 333) reaching levels of 8.6 Bodansky units per 100 cc. In 4 instances the values were within normal limits despite the presence of marked congestive hepatomegaly.

TABLE VII

Summary of analyses of the blood in nine cases of liver abscess, ten cases of chronic passive congestion of the liver, eight cases of miscellaneous disorders of the liver, and nine cases of gall bladder disease with indeterminate effect upon the patency of the common bile duct

Number	Sex	Age	Diagnosis	Date	Serum		Remarks
					Phosphatase	Bil. rubin	
		years			Bodansky units per 100 cc.	mgm per 100 cc.	
A. LIVER ABSCESS							
323	♂	59	Ruptured viscus? (operation)	May 29 1939	42.1	4.0	Sub- and intrahepatic abscess at hilum. Jaundiced one week.
324	♂	21	Miliary tuberculosis (autopsy)	July 26, 1938	26.6	4.7	Disseminated tubercular abscesses of liver. Jaundiced one week.
325	♀	27	Septic (autopsy)	August 9 1938	18.0	11.5	Large hilar abscess obstructing main hepatic ducts. Jaundiced two years, intermittently.
				September 22 1937	15.2	7.0	
				November 16, 1937	49.4	3.5	
				December 9, 1937	39.6		
				December 20, 1937	34.5	2.9	
326	♀	52	Post-operative infection (operation)	November 23 1938	14.0	0	Multiple liver abscesses.
327	♀	26	Tuberculosis? (autopsy)	October 13 1937	13.4	trace	Multiple intra- and subhepatic abscesses.
				October 22, 1937	17.0	0	
				January 10 1938	19.1	3.9	
328	♂	32	Multiple liver abscesses (operation)	December 11, 1937	10.3	3.0	Following empyema of gall bladder.
329	♂	70	Amebic abscess (clinical)	December 28, 1937	7.3	3.0	
				November 1 1937	9.5	2.1	Amebic cysts in stool fever etc.
				January 19 1939	8.2	0	Recurrence.
330	♂	26	Amebic abscess (operation)	May 17 1939	8.1	2.0	Recurrence.
331	♂	44	Amebic abscess (clinical)	January 28, 1938	7.9	0	Amebias in stool fever etc.

B CHRONIC PASSIVE CONGESTION OF THE LIVER
(Autopsy diagnoses in every instance)

332	♂	52	Myocardial infarctions	March 23 1938	8.6	1.0	Terminal Friedlaender pneumonia.
333	♂	53	Arteriolosclerosis	January 19 1937	8.6	2.0	
334	♀	49	Myocardial infarctions	February 3, 1938	8.2	2.0	
				November 21 1938	6.1	trace	
335	♀	48	Rheumatic valvular disease	March 10 1937	8.0	3.2	
336	♀	16	Rheumatic pericarditis	January 22, 1934	7.1	0	
337	♂	37	Rheumatic pericarditis	April 22, 1937	6.7	2.5	
338	♂	15	Rheumatic pericarditis	October 21, 1938	3.9	8.3	
339	♂	38	Bacterial endocarditis	October 28, 1937	2.5	0	
340	♂	41	Bacterial endocarditis	November 14 1938	2.5	0	
341	♂	60	Arteriolosclerosis	December 11, 1937	2.3	1.0	

TABLE VII—Continued

Number	Sex	Age	Diagnosis	Date	Serum		Remarks
					Phos- phatase	Bill- rubin	
		years			Bodansky units per 100 cc.	mgm per 100 cc	
C MISCELLANEOUS DISORDERS OF THE LIVER							
342	♀	75	Stone in common duct (opera- tion)	June 18 1938	20.0	2.0	Localized Paget's disease pelvis
343	♂	64	Stone in common duct (opera- tion)	March 5 1935	17.9	3.0	Localized Paget's disease pelvis
344	♂	48	Pneumonia, septicemia (autopsy)	January 6 1937	4.8	9.0	Complicated by jaundice.
345	♂	26	Pneumonia (clinical)	March 26 1937	1.7	2.8	Complicated by jaundice.
				April 2 1937	5.0	2.0	
				April 13 1937	4.0	0	
346	♂	70	Pneumonia empyema, uremia (autopsy)	January 18 1937	7.7	6.9	Complicated by jaundice
				February 8 1937	12.9	1.0	
				February 10 1937	22.4	1.0	
347	♀	58	Tuberculosis of liver (liver biopsy)	January 28 1939	14.4	2.0	Extensive infiltration
348	♂	35	Hodgkin's disease (autopsy)	June 9 1936	7.3	9.0	Extensive infiltration of portal areas
349	♀	28	Echinococcus cyst of liver (operation)	September 6 1938	4.6	trace	Left lobe of liver involved
				March 17 1939	4.5	trace	
D CHRONIC GALL BLADDER DISEASE							
350	♀	54	Cholelithiasis (operation)	October 30 1937	19.6	15.0	Operation December 7 1937 no stone in com- mon duct
				November 13 1937	10.0	9.0	
				November 22 1937	7.5	2.9	
351	♀	63	Cholelithiasis (operation)	May 25 1937	18.0	6.0	Operation June 8 1937 no stone in common duct
				May 29 1937		1.0	
352	♀	50	Cholelithiasis (operation)	March 6 1938	11.9	6.0	Operation March 14 1938 no stone in common duct.
				March 8 1938	8.7	trace	
353	♀	40	Cholelithiasis (operation)	September 29 1938	7.3	7.5	Operation October 4 1938 no stone in common duct
354	♀	19	Cholelithiasis (operation)	October 2 1936	3.0	8.3	Operation October 14 1936 no stone in com- mon duct
				October 5 1936		3.2	
355	♀	61	Cholelithiasis (operation)	March 13 1936	3.6	trace	Operation March 16 1936 no stone in common duct
356	♂	56	Cholelithiasis (operation)	March 20 1939	4.4	trace	Operation March 25, 1938 no stone in common duct
357	♀	59	Cholelithiasis (operation)	May 26 1939	3.6	0	Operation June 6 1939 no stone in common duct
358	♂	30	Cholelithiasis (operation)	March 7 1939	6.5	6.2	Operation March 16 1939 no stone in common duct, spasm of sphincter of Oddi?
				March 13 1939	5.7	11.5	
				March 20 1939	2.3	17.0	
				March 29 1939	7.2	6.3	
				April 10 1939	5.4	2.7	

Eight patients with miscellaneous types of hepatic disease are included in Table VIIC. Cases 342 and 343 illustrate difficulties in the application of the serum phosphatase determination to the differential diagnosis of jaundice in patients with bone disease. In both instances a marked increase in serum phosphatase activity, with slight jaundice, was noted but was not interpretable because Paget's disease was present (localized lesions not apparent clinically but disclosed incidentally by x-rays). These patients subsequently proved to have stone in the common duct. In a previously recorded instance (Case 116) confusingly high serum phosphatase values due to clinically unrecognizable Paget's disease were obtained in a case of hepatitis.

Jaundice developing in patients with pneumonia ("toxic" or "infective" jaundice) usually is as-

sociated with comparatively little increase in serum phosphatase activity, as shown in Cases 344 and 345. In Case 346, whose course was complicated by empyema, there was a terminal rise in the serum phosphatase activity to 22.4 Bodansky units per 100 cc, a level we have not encountered otherwise in this type of case. There was no adequate explanation for this increase at autopsy, which disclosed only focal necroses of the liver. Case 347, with serum phosphatase of 14.4 Bodansky units, was found to have tubercles extensively involving the liver. Case 348 had Hodgkin's disease of the liver, confined to the portal areas. Case 349 is of interest because a very large echinococcus cyst was present but did not effect a significant rise in serum phosphatase activity. The cyst involved the left lobe of the liver, which may have some bearing on the result.

Table VIID comprises 9 cases of gall bladder disease with jaundice, a large and important clinical group but one which presents many difficulties in classification. Several investigators in this field have regarded all such cases as jaundice due to obstruction of the common bile duct, irrespective of whether or not operation disclosed a stone in the common duct, apparently on the assumption that mechanical obstruction or sphincter spasm are the sole causes of jaundice under these circumstances. So far as evaluation of the serum phosphatase determination is concerned, we have preferred to classify as obstructive jaundice only those cases of biliary tract disease in which mechanical obstruction of the common bile duct could be demonstrated at operation or autopsy. Patients in whom no such obstruction was found have been regarded for the present purpose as unclassifiable and were omitted from our statistical series. In some of our patients in this category (Cases 350, 351 and 352) serum phosphatase values over 10 Bodansky units were associated with definite hyperbilirubinemia and typical biliary colic suggesting stone in the common bile duct, there was spontaneous cessation of pain and rapid subsidence of jaundice, and operation about 1 week thereafter disclosed cholelithiasis but no stone in or dilatation of the common duct. This sequence of events is consistent with spontaneous passage of a stone in the common duct but proof is wanting. In other instances (Cases 353 and 354) the clinical course and the operative findings were the same definite jaundice was present but the serum phosphatase level showed little or no increase. It is impossible to say whether a stone occluded the common duct at the time the patient was icteric and the serum phosphatase failed to rise to its usual level, or whether the jaundice was caused by transitory infection of the biliary tract or hepatitis. Even more difficult of interpretation are instances in which the first serum phosphatase determination was made after jaundice had subsided spontaneously (Case 355) conditions under which the method is without value. Cases 356 and 357 illustrate essentially normal serum phosphatase values characteristic of chronic gall bladder disease uncomplicated by jaundice.

Another source of error inherent in our classification of obstruction of the extrahepatic biliary

tract on the basis of mechanical occlusion lies in the possible inadvertent exclusion of obstruction due to spasm of the sphincter of Oddi. In those instances in which this mechanism was suggested at operation as the cause of icterus, it was not possible to obtain convincing objective proof of the causal relation of spasm to jaundice. For example Case 358 was found to have cholecystitis and cholelithiasis but no stone in or dilatation of the common bile duct at operation (at which time the serum bilirubin was markedly elevated but the serum phosphatase activity insignificantly increased). Cholangiograms showed some narrowing of the distal end of the common duct, without delay in emptying. Liver biopsy was variously interpreted as hepatitis or essentially normal liver but was not thought to suggest obstructive jaundice. Manometer readings of bile duct pressure after injection of saline into the drainage tube gave equivocal results. The jaundice increased after operation, then slowly subsided.

ANALYSIS AND DISCUSSION OF TOTAL SERIES OF OBSERVATIONS 1933 TO 1939

1 Distribution of serum phosphatase values in major diseases of the liver and biliary tract

Perhaps the clearest summary of our results is afforded by an analysis of their distribution in certain common disorders of the liver and biliary tract. The total number of such cases available for analysis is 308 the several categories of disease considered and their respective representation being indicated in Table VIII. For reasons stated elsewhere observations in children are excluded. Only the initial values obtained in each patient were employed for purposes of analysis but an exception was made in Case 246 for reasons evident in the text. Patients with biliary cirrhosis (including Case 73 (1)) are classified according to the type of biliary tract obstruction and do not appear in the cirrhosis group.

Of a total of 34 patients with jaundice due to stone obstructing the common bile duct 5 or roughly 15 per cent had serum phosphatase levels less than 10 Bodansky units per 100 cc. as did 2 or roughly 5 per cent of 45 patients with non-calculous common bile duct obstruction (chiefly carcinoma of the head of the pancreas) C 1

TABLE VIII

Distribution of serum phosphatase values in major disorders of the liver and biliary tract, analysis of results in 308 adults (initial values only)

Diagnosis	Total number of cases	Distribution of cases by phosphatase values				
		Zone of normal values	Zone of indeterminate diagnostic significance			
				<4.0	4.1-9.0	9.1-12.0
				12.1-25.0	>25.0	Bodansky units per 100 cc.
Stone in common bile duct	34	0	5	5	21	3
Non-calculous obstruction of common bile duct	45	0	1	5	25	14
Catarrhal jaundice	69	8	48	8	4	1
Jaundice after hepatotoxic drugs	38	2	18	4	13	1
Hemolytic jaundice	13	13	0	0	0	0
Hepatic cirrhosis (non-biliary)	44	14	20	5	4	1
Neoplastic involvement of liver	47	4	18	5	18	2
Liver abscess	10	0	2	3	3	2
Chronic passive congestion of liver	8	3	5	0	0	0

ering the 79 cases of proven obstructive jaundice as a whole, about 10 per cent failed to show phosphatase levels over 10 Bodansky units per 100 cc serum. Of a total of 107 cases classified as "hepatitis," 82 or roughly 75 per cent had serum phosphatase levels less than 10 Bodansky units per 100 cc. If, as we have proposed, patients with jaundice following exposure to hepatotoxic agents be excluded from the heterogeneous "hepatitis" group, then 62 of the 69 remaining cases (90 per cent) had serum phosphatase levels less than 10 Bodansky units per 100 cc.

There would seem to be little point in further pursuing the analysis along these lines because, in practice, many values that are slightly above or below 10 Bodansky units are of indeterminate diagnostic significance. This difficulty is recognized in the distribution of values as summarized in Table VIII, which gives a more representative picture of the applicability of the serum phosphatase determination in our hands. It will be noted that 6 of 79 patients with proven common duct obstruction had serum phosphatase levels not exceeding 9.0 Bodansky units per 100 cc, 5 being instances of choledocholithiasis with incomplete or intermittent occlusion. The values in 10 patients of this group further fell into the indeterminate zone of 9.1 to 12.0 Bodansky units

Sixty-three or 80 per cent of the total number of cases with jaundice due to gross common duct obstruction showed serum phosphatase values over 12.0 Bodansky units per 100 cc. Values over 25 Bodansky units were obtained usually but not invariably in patients with complete and protracted obstruction of the common bile duct due to neoplasm.

In "catarrhal" jaundice, on the other hand, 56 of 69 cases (about 80 per cent) had serum phosphatase levels less than 9.0 Bodansky units per 100 cc, 8 were at indeterminate levels and 5 (about 7 per cent) exceeded 12.0 Bodansky units. When the relation of serum phosphatase to serum bilirubin levels in these two major types of jaundice is taken into consideration, the differences between them become even more striking. Those patients with obstructive jaundice who showed little increase in serum phosphatase activity had relatively slight hyperbilirubinemia, as obstruction was incomplete, whereas even marked jaundice of hepatogenous origin was associated for the most part with comparatively little elevation in serum phosphatase activity.

The distribution of serum phosphatase values in hemolytic jaundice and in chronic passive congestion of the liver is limited to the normal range or slightly above, as already indicated. In patients with liver abscess the values are preponderantly in the range of obstructive jaundice or fall within the zone of indeterminate significance. Thirty-four of 44 cases (about 75 per cent) of cirrhosis other than biliary cirrhosis had serum phosphatase levels less than 9.0 Bodansky units per 100 cc, and 5 fell within the indeterminate zone.

The distribution of serum phosphatase values in two categories of liver disease deserves special comment because a double peak in the incidence curve appears, one within the "hepatitis" zone, the other in the zone of obstructive jaundice. These are the group with jaundice due to hepatotoxic drugs and the series comprising patients with neoplastic involvement of the liver. In the former, as already indicated, there is some evidence that two distinct types of jaundice may be present. In the latter group, the spread in serum phosphatase values appears to relate to the number and distribution of metastases in the liver.

2 *Clinical usefulness and limitations of the determination of serum phosphatase activity as applied to diseases of the liver and biliary tract*

A Evaluation of the method as a supplementary aid to the clinical differentiation of the several types of jaundice The chief usefulness of the determination of serum phosphatase activity derives we believe, from the consistency with which distinctly increased values accompany obstruction of the extrahepatic biliary tract. We have found it helpful to recognize that jaundice in patients with serum phosphatase levels less than 10 Bodansky units per 100 cc. is probably not due to gross obstruction of the common bile duct. When exploration was contemplated in such patients, it proved good policy to observe the patient further until additional clinical and laboratory data could be obtained.

While the absence of significantly increased serum phosphatase activity in icteric patients is valid evidence against obstruction of the common bile duct in our experience, it does *not* follow that a serum phosphatase value greater than 10 Bodansky units accompanying jaundice automatically marks the patient for exploration. The finding of a serum phosphatase level exceeding 10 Bodansky units per 100 cc. in icteric patients, to be sure, is consistent with the clinical diagnosis of obstructive jaundice. But in addition to obstruction of the extrahepatic biliary tract warranting surgical intervention, marked elevations in serum phosphatase activity also occur frequently in arsenphenamine jaundice and with liver metastases occasionally in hepatitis and other liver diseases requiring conservative management and in a variety of skeletal disorders. It was sometimes but not always possible for us to exclude these further possibilities by careful history taking and appropriate roentgenographic and clinical studies.

To indicate what seems to us to be a particular advantage of the serum phosphatase determination in the differential diagnosis of jaundice requires a consideration of certain difficulties inherent in the clinical application of any physiological aid to the diagnosis of liver disease. As pointed out by several investigators (8-9) the simple but arbitrary division of clinical jaundice into three discrete categories obstructive hepato-

genous and hemolytic, however convenient clinically, does not adequately define the mechanisms actually operating in many patients. The initial or predominant cause of jaundice may fall into one or another of these distinct categories but serious disturbance of one mechanism soon involves others and this complexity is reflected in equivocal or confusing results with the test employed. The disadvantage of most liver function tests used in the differential diagnosis of jaundice lies in the circumstance that they provide only negative evidence for obstruction—that is, mechanical obstruction of the common bile duct is suggested by the *absence* of any significant alteration in a given function of the liver in patients with jaundice. While this often suffices if significant liver parenchymal injury complicates obstruction (as with cholangitis or biliary cirrhosis developing in protracted obstructive jaundice) then the negative effect of obstruction may be superseded by the positive effects of secondary inflammatory or degenerative changes. Under these circumstances, liver function tests not infrequently give equivocal or confusing results and the possibility of error is not minimized by a multiplicity of such tests all having much the same limitation.

It would seem desirable rather to complement the liver function tests with some positive measure of biliary tract obstruction. We find the determination of serum phosphatase activity satisfactory for this purpose since inflammatory or cirrhotic changes secondary to common bile duct obstruction do not ordinarily significantly lower the phosphatase level of the serum. Other positive indices of biliary tract obstruction such as the total cholesterol content of the serum have proved less consistently helpful in our hands (Table I) particularly in the clinically important group of patients with incomplete mechanical obstruction of the common bile duct. The range of variation in the serum cholesterol content of different normal subjects moreover is extremely broad so that interpretation of values in any one case of jaundice may be difficult, whereas we find the range of variation in serum phosphatase activity in the normal adult relatively narrow and the many-fold increase encountered in obstructive jaundice facilitates interpretation of results.

There are further minor advantages that recommend the serum phosphatase determination for

test purposes in jaundiced patients it does not require the administration of substances that may or may not be innocuous to the disordered liver, and it is not significantly affected by renal disease, in our experience

As to the disadvantages of the method, there are several that limit its applicability in the differentiation of the several types of jaundice. The foremost of these is overlapping of serum phosphatase values in the obstructive and hepatogenous groups of jaundiced patients. The degree of overlapping noted by different investigators has varied extraordinarily, some finding it so large as to disqualify the method for differential diagnosis (3b, 3f), others finding the overlapping of values sufficient to limit but not invalidate the usefulness of the determination (3c, 3h). There is general though not unanimous agreement, however, regarding the relative consistency of distinctly increased serum phosphatase levels in jaundice due to obstruction of the common bile duct. As suggested elsewhere (1), these discrepancies in experience may be ascribable, in part, to variations in technique of the serum phosphatase determination, in the classification of clinical material, or in the adequacy of evidence adduced for the classification of cases.

The differentiation of obstructive from hepatogenous jaundice is further confused by overlapping of serum phosphatase values obtained in other diseases of the liver and biliary tract, as shown in our tables. Consequently, we feel that the chief usefulness of the determination in the differential diagnosis of jaundice is in excluding, with high probability, obstruction of the common bile duct as a cause of jaundice in patients showing no marked increase in serum phosphatase activity.

Another difficulty with the serum phosphatase determination as applied to the differential diagnosis of jaundice lies in its lack of specificity. As is well known (10, 11, 12, 13), the phosphatase activity of the serum is increased in a variety of skeletal disorders, notably Paget's disease, rickets, carcinoma with bone metastases, particularly of the osteoplastic type, hyperparathyroidism and osteogenic sarcoma. The serum phosphatase level cannot be applied to the diagnosis of disease of the liver or biliary tract in patients presenting any of these bone conditions. Ordinarily, apprecia-

tion of this limitation avoids confusion because the skeletal disorder is readily recognizable. But errors occur occasionally when localized Paget's disease or metastatic bone involvement, discoverable only by appropriate x-rays, is overlooked. Apart from these skeletal disorders, no other conditions significantly affect the serum phosphatase level consistently enough to interfere seriously with the use of the method in jaundice, so far as we could discover. If skeletal disease can be ruled out, it has been our experience that patients with definitely elevated serum phosphatase level, whether jaundiced or not, generally prove to have one or another type of liver disease.

Further difficulties with the serum phosphatase determination are encountered when it is applied to the differential diagnosis of jaundice in children. The method appears to be of no value in an important problem of this kind in infants: the differentiation of congenital atresia of the bile ducts from "physiological jaundice." Throughout the period of skeletal growth, the range of normal variation in serum phosphatase activity is so broad and ill-defined (roughly 4 to 12 or more Bodansky units per 100 cc), that the values obtained in hepatitis in children are confusingly high (3a, 3g). Although our experience with this age period has been limited, we feel that for the present the method is not applicable to growing children.

Finally, as already indicated, we find the serum phosphatase determination of little value in the diagnosis of Laënnec's cirrhosis and of largely academic interest in hemolytic jaundice.

B The determination of serum phosphatase activity as an aid in the diagnosis of complications following surgery of the biliary tract. In all, 24 patients with obstructive jaundice were studied both before and after attempted relief of common duct obstruction by surgical measures. Because of the complexities involved, our data are too incomplete for any but the most general inferences, and in 3 patients are wholly inadequate for analysis.

Decompression by establishment of external or internal biliary fistulae in obstructive jaundice, if successful in effecting adequate drainage and if not complicated by infection, resulted in a roughly parallel fall in both serum bilirubin and phosphatase levels (Cases 5, 11, 18, 21, 23, 125, 128, 151,

155) The post-operative course under these circumstances was usually uneventful. When exploration disclosed that obstruction of the common bile duct was due to carcinoma so extensive as to discourage any attempt at drainage, or when an unsuccessful attempt at drainage was made, both serum bilirubin and phosphatase showed a parallel tendency to remain elevated or to rise further (Cases 2, 22, 168).

In many patients both serum bilirubin and serum phosphatase levels fell following establishment of biliary drainage but a secondary rise in serum phosphatase activity was noted, not necessarily associated with a comparable rise in serum bilirubin. Meranze, Meranze and Rothman (3h) called attention to this phenomenon and pointed out that it occurred usually when convalescence was stormy, an observation that coincides in general with our experience. The significance of this secondary rise in serum phosphatase activity appears to depend largely upon the nature of the obstruction and the type of operation performed. In patients with stone in the common duct the principal cause was infection such as subphrenic abscess involving the hilum of the liver (Case 133) or cholangitis (Case 138). In patients with carcinomatous obstruction of the common duct, a late recurrence of serum phosphatase activity after establishment of internal biliary fistulae was associated with cholangitis leading to closure of the stoma and to multiple liver abscesses (Case 10) with the development of liver metastases (Case 162) or with cholangitis and liver metastases (Case 21). The association of increased serum phosphatase activity with complications attending the development of protracted biliary fistulae after cholecystectomy has already been illustrated by three case summaries.

Although our data are incomplete, there would appear to be a general correlation between the post-operative serum phosphatase level and the success of surgical procedures in the establishment of adequate biliary drainage. In this sense serum phosphatase determinations may aid in the anticipation of certain post-operative complications, particularly in the development of cholangitis or of liver metastases (the serum bilirubin level being an uncertain guide). Limitations in the use of the determination for this purpose are illustrated by instances in which con-

valescence was without serious incident yet the fall in serum phosphatase was either very slow (Case 17) or was interrupted by a slight rise without apparent cause (Case 126). One patient (Case 139) who developed peritonitis post-operatively, showed a fall in serum phosphatase activity associated with a distinct rise in serum bilirubin. Necropsy disclosed extensive liver necrosis suggesting 'toxic' hepatitis.

C Serum phosphatase determination as a means of early detection of metastases in malignancy. The liver is a site of predilection for distant metastases in many types of tumor but because of difficulties in the early detection of liver metastases by any means short of exploration, the recognition of metastatic liver involvement is frequently delayed until palpable nodules become obvious. Jaundice as is well known, is a late and inconstant manifestation of metastatic malignancy. It has been our experience that usually before there is any demonstrable liver enlargement, the serum phosphatase activity of patients with liver metastases often shows a distinct rise (14, 12, 1). On a number of occasions, the increased serum phosphatase level was the only objective evidence we could obtain of the presence of metastatic liver involvement demonstrated by exploration or necropsy shortly thereafter. However, the absence of a significant rise in the serum phosphatase level does not exclude the possibility of metastatic liver involvement since some patients with essentially normal serum phosphatase levels nevertheless proved to have liver metastases (1, 12, 13, 15) usually a few small nodules but sometimes more extensive invasion. Apparently, chance variations in the distribution and size of metastases affect the reliability of the method particularly when few nodules are present, and limit the usefulness of the serum phosphatase determination for this purpose. Moreover, if jaundice is absent it is necessary to exclude metastatic involvement of the skeleton (usually but not always possible by x ray) before concluding that increased serum phosphatase activity is due to liver metastases though it is often immaterial as when ablation of a primary tumor is contemplated whether secondaries involve liver or skeleton, so long as they can be detected early.

It is our impression that, as emphasized by Meranze, Meranze and Rothman (15), valid early

evidence for metastases may be afforded by the finding of a definitely elevated serum phosphatase level in patients known to have malignancy. This obviously does not apply if other causes of increased serum phosphatase activity (Paget's disease, etc.) are present or if the primary tumor itself may be responsible for such an increase (osteogenic sarcoma, primary tumors obstructing the biliary tract). It should be kept in mind that the serum phosphatase level is not affected by metastases to such organs as lungs and may not be significantly affected by either liver or bone metastases (particularly of the osteolytic type) or both (see Case 320).

3 *Mechanisms regulating the behavior of the serum phosphatase level in disease of the liver and biliary tract*

According to the prevailing concept (10), "alkaline" serum phosphatase³ originates for the most part in bone-producing cells (an assumption based chiefly upon the high phosphatase activity of bone) and, after escaping into the circulating fluids, is excreted in the bile (an assumption based upon the high phosphatase activity of the bile). Clinical experience, on the whole, is in good agreement with this theory. Serum phosphatase determinations in a wide variety of diseases have shown, empirically, that appreciable elevations in serum phosphatase activity are observed consistently in only two general pathological states: (1) diseases of the skeletal system in which there is active, widespread formation of bone or cartilage (11, 12, 13, 17), (2) diseases of the liver and biliary system in which there is obvious or presumptive impingement upon the excretory channels (biliary tract), whether extra- or intrahepatic. That skeletal disorders characterized by increased osteogenesis cause elevated serum phosphatase levels is understandable, on the basis of the experimental evidence for the osseous origin of "alkaline" phosphatase, as due to increased formation of the enzyme. That hepatic disorders of the obstructive type cause increased serum phosphatase activity would be anticipated as a result of retention of the enzyme

due to blocking of its excretory channel, the biliary tract. The prevailing concept therefore affords an explanation for the occurrence of a common phenomenon (increased serum phosphatase activity) in diseases that are otherwise extremely heterogeneous in character.

There is a diversity of opinion regarding the validity of the "retention" theory as applied to the increased serum phosphatase activity observed in some hepatic disorders. It is wholly probable that even if blocking of the excretory biliary channels results in retention of phosphatase in the serum, other factors also affect the serum phosphatase level in obstructive jaundice. It is difficult otherwise to explain the wide range in serum phosphatase activity in different patients each having complete and protracted obstruction of the common bile duct (from 113.1 to 9.6 Bodansky units per 100 cc in our cases of carcinoma of the head of the pancreas). Evidently, the level of serum phosphatase at which equilibrium is re-established in the blood following common duct obstruction does not depend solely upon the degree of obstruction. It would seem likely that the rate of phosphatase formation and the adequacy of other methods of disposing of serum phosphatase (by mechanisms as yet unknown) play a rôle here. That these other factors are operative does not imply, however, that the "retention" theory is invalid.

Several alternative explanations have been offered in lieu of the "retention" theory. Thannhauser and his associates have interpreted their clinical and experimental studies (18, 19) to signify that the increased serum phosphatase activity observed in sundry diseases, including obstructive jaundice, is due not to increased concentration of the enzyme in the blood but rather to the effect of an activator on the enzyme. This hypothesis is based upon their observation that ascorbic acid activates serum phosphatase (20), a phenomenon subsequent studies (21, 22) proved to be an artefact. The activity of "alkaline" phosphatase is slightly inhibited by bile salts (23).

Another explanation for the behavior of the serum phosphatase level in diseases of the liver and biliary tract lies in the possible hepatogenous origin of at least part of the "alkaline" phosphatase content of serum. Bodansky has shown (24) that nutritional factors may influence the

³ For the sake of simplicity we have referred to the serum phosphatases as "phosphatase." In reality, at least one "alkaline" and at least one "acid" phosphatase system is present in serum.

'alkaline' phosphatase activity of the serum and has raised the question of extra-osseous sources of the enzyme (25). Liver tissue is known to contain appreciable amounts of both "alkaline" and 'acid' phosphatases. It is possible that the behavior of the serum phosphatase in hepatic disease is due not to retention of a phosphatase of osseous origin but to varying effects of hepatic disease upon the elaboration and secretion of phosphatase by the liver parenchyma. Much of the evidence now available both experimental and clinical, is interpretable either way. Current discussion along these lines is reminiscent of the controversy which prevailed regarding the hepatic vs. extra hepatic origin of the bile pigments until incontrovertible proof of their chiefly extra-hepatic origin was obtained (26).

No such definitive evidence is now available one way or the other, concerning the validity of the "retention" theory of the behavior of serum phosphatase levels in disease of the liver and biliary tract. Such evidence must be obtained by proper experiment. But so far as clinical observations go the distribution of our data on adults (Table VIII) is consistent, on the whole with the "retention" theory. Gross obstruction of the common bile duct quite regularly results in markedly increased serum phosphatase activity, the exceptions occurring chiefly with incomplete or intermittent obstruction. Interposition of masses in the liver substance (tumor nodules liver abscess etc.) that might block off major intrahepatic biliary passages likewise leads to elevated serum phosphatase values. Hepatogenous or "catarrhal" jaundice usually causes relatively little increase in serum phosphatase activity as compared with the degree of hyperbilirubinemia not more than might well be due to varying intrahepatic obstruction of the finer biliary radicles. Hemolytic jaundice does not ordinarily affect the serum phosphatase level. The varying serum phosphatase levels observed in jaundice due to hepatotoxic drugs accord with other criteria in patients with high levels and are all indicative of the possibility of intrahepatic biliary obstruction. Fibrosis of the portal areas (or about the central veins) seems not to disrupt the biliary tract in most instances judging by the preponderance of cases with little or no jaundice and comparatively little increase in serum phosphatase activity ap-

parently new bile duct proliferation maintains the integrity of the excretory channels. But when disorganization of the biliary tract with jaundice, occurs in Laennec's cirrhosis (due to complications like infection or neoplastic degeneration), the serum phosphatase level rises.

On the other hand, it is clear that markedly increased serum phosphatase activity may occur with little or no hyperbilirubinemia (as for instance with metastatic liver malignancy) and this dissociation has seemed to some (27, 28) incompatible with the 'retention' theory both phosphatase and bilirubin being constituents of bile obstruction of the biliary tract sufficient to cause retention of phosphatase ought to be associated with a comparable degree of bilirubin retention. This would follow of course if the blood levels of both bilirubin and phosphatase depended solely upon their common rate of excretion in the bile. However bile pigments are excreted in the urine when there is biliary obstruction with retention of bile in the blood whereas the human kidney appears to be impermeable to 'alkaline' serum phosphatase.⁴ This difference with respect to renal excretion affords a possible explanation for the occurrence of increased serum phosphatase activity with little or no jaundice a dissociation which does not seem to us to disqualify the retention theory. In hepatitis the absence of appreciable increase in phosphatase activity despite marked hyperbilirubinemia due to blocking of bile pigments by the disordered parenchyma, suggests possible shunting of the enzyme around the polygonal cells.

The increased serum phosphatase activity sometimes observed in patients with external biliary fistulae draining bile likewise appears to be incongruous with the 'retention' theory since under these circumstances bile and therefore

⁴ That is, the "alkaline" phosphatase activity of human urine is negligible in jaundiced (or normal) human subjects. Interestingly enough, in the cat, the one species in which "alkaline" phosphatase is known to be present in appreciable amounts in the urine (29) ligation of the common bile duct causes little or no rise in serum phosphatase activity (30).

In passing it might be noted that dissociation of serum bilirubin and phosphatase is not peculiar to phosphatase among bile constituents. For example, a similar dissociation between serum bilirubin and total cholesterol values is the rule in hepatogenous jaundice.

phosphatase, is certainly being excreted. The validity of this inconsistency would seem to depend upon whether or not the excretion of phosphatase in a given instance is adequate, always a difficult matter to appraise. The fairly consistent occurrence of clinical complications, when such dissociation was observed by us, leads to the suspicion that drainage under those circumstances may not be as adequate as would appear. It is interesting to note in this connection that patients with seemingly free drainage of bile through external fistulae may show other peculiarities such as increased serum cholesterol (31) and rather marked increase in bromsulfalein retention (9).

A more convincing inconsistency of the "retention" theory encountered in our clinical material, however, was that presented by infants with jaundice due to congenital atresia of the bile ducts. In spite of typical obstructive jaundice, the serum phosphatase level in this condition has been found to be within or only moderately above the normal range in infants. We obtained values of 11.1 and 6.3 Bodansky units per 100 cc respectively in 2 such previously recorded instances (Cases 112 and 113, see Donovan (32) for clinical details). Similar results have been obtained by other investigators (3f, 3i). Another case reported here (Case 173) showed a level of 17.1 Bodansky units, a value which we regard as definitely elevated but probably less than might be anticipated in obstructive jaundice at this age period. It remains to be determined whether these unexpected results signify that the "retention" theory is untenable or whether they are ascribable to some as yet unknown peculiarity of the phosphatase metabolism in the infant.

SUMMARY

Our experience during the period 1933 to 1939 with serum phosphatase determinations (Bodansky method) in disorders of the liver and biliary tract is summarized. An analysis was made of the results in over 300 adult patients in whom the diagnosis, apart from the hepatitides, could be established at operation or autopsy.

Of 79 adults with proven obstruction of the common bile duct, 72 (roughly 90 per cent) had serum phosphatase levels more than 10 Bodansky units per 100 cc. In the "hepatitis" group, 82

of 107 adult cases had serum phosphatase levels less than 10 Bodansky units per 100 cc, but if, as we have proposed, patients with jaundice following exposure to hepatotoxic agents be segregated from the "catarrhal" jaundice group, then 62 of the 69 latter cases (roughly 90 per cent) had serum phosphatase levels less than 10 Bodansky units per 100 cc. There is a striking dispersion in the serum phosphatase values of patients with jaundice following exposure to hepatotoxic agents, especially arsphenamine. This dispersion may have clinical significance.

Hemolytic jaundice was associated with normal serum phosphatase values. Of 44 patients with advanced cirrhosis (exclusive of biliary cirrhosis), 34 had serum phosphatase values less than 9 Bodansky units per 100 cc. Forty-seven patients with proven neoplastic involvement of the liver showed a wide dispersion in serum phosphatase levels, depending apparently upon the number and location of metastases. Chronic passive congestion of the liver caused little or no elevation in serum phosphatase activity, liver abscess was usually associated with distinctly increased values.

We find the determination of serum phosphatase activity of limited but definite value as a supplementary aid to the clinical differentiation of the several types of jaundice. It is useful particularly in ruling out obstruction of the extra-hepatic biliary tract, an improbable cause of jaundice in patients with serum phosphatase values less than 10 Bodansky units per 100 cc. When applied to the differential diagnosis of jaundice, the following sources of error should be recognized: (a) overlapping of values in the obstructive and hepatogenous groups of jaundice (about 10 per cent in either direction, in our experience), (b) unspecificity, as certain skeletal disorders increase serum phosphatase activity, (c) inapplicability to jaundice in children, particularly in congenital atresia of the bile duct.

The determination of serum phosphatase activity may be useful also in anticipating certain complications following surgery of the biliary tract. Pre- and post-operative determinations in 24 patients with obstructive jaundice disclosed a distinct correlation between post-operative serum phosphatase trends and the post-operative clinical course.

Furthermore, the determination of serum phosphatase activity is of some value in the relatively early detection of liver or skeletal metastases in patients known to have malignancy

Possible mechanisms regulating the serum phosphatase level in disorders of the liver and biliary tract are considered, with special reference to the "retention" theory. To judge from our empirical clinical experience, the serum phosphatase level in the adult is a sensitive criterion of the integrity of the excretory biliary channels, extra- and intra hepatic, affording positive evidence for obstruction. It is suggested that the determination be employed to complement "liver function" tests, which measure impairment of a given function of the liver parenchyma but provide merely negative evidence for obstruction, and the dye retention tests which are inapplicable in jaundiced patients

We are indebted to the many members of the Medical and Surgical Staffs who permitted study of their cases.

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METABOLIC DISTURBANCES IN EXPERIMENTAL HUMAN VITAMIN B DEFICIENCY

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Recent animal experiments have indicated that the vitamin B complex is necessary for the normal metabolism of carbohydrate. The abnormality most frequently reported in vitamin B₁ deficiency is an interference with some phase of the oxidation of carbohydrate, so that intermediary products of glucose metabolism accumulate in the tissues (1, 2, 3, 4, 5) and in the blood (6) while tissue oxidation decreases. The substances most commonly observed to accumulate are lactic acid, pyruvic acid and certain as yet unidentified compounds which have in common the ability to bind bisulphite. Many other phenomena are reported with far less consistency in animals deficient in various members of the B complex such as the occurrence of hyperglycemia (7), ketonuria and interference with the storage of glycogen in the liver (8, 9, 10) decrease in the respiratory quotient (11), sensitivity to insulin (12) etc. The application to man of the knowledge gained from these animal experiments must however be made cautiously for several reasons. Different species of laboratory animals vary greatly in their requirements for the vitamin B group, so that it is uncertain in how far particular data obtained from animals are transferable to man. The animal experiments have furthermore been performed under specialized laboratory conditions which permit the study of one or more isolated members of the vitamin B complex. Although this form of study provides important data concerning the function of separate fractions of the B complex it has recently been shown that deficiency of one fraction so modifies the manifestations of deficiency due to other members of the group (13) that the effects of combined deficiencies cannot justifiably be assumed from

study of isolated fractions. It is probable that such experiments fail to give accurate information concerning the complex conditions of multiple deficiency observed in man. Unfortunately the study of fully developed human deficiency as seen in the clinic is unsatisfactory because the exact nature and extent of the deficiency cannot be determined with accuracy, and unknown factors undoubtedly influence the phenomena under consideration. If the problem as applied to man, is to be clarified, it seems desirable that studies be made directly upon human beings living under controlled conditions of vitamin B deficiency² in whom the influence of deficiency of the B complex as a whole may be compared with effects of deficiency of the separate fractions. The present paper is a report of such a study made upon one individual who voluntarily consumed for a period of 4 months a constant diet deficient only in the B complex. The fact that this woman consumed a constant amount of food throughout observation permitted a more accurate study of the effects of deficiency of the B complex alone than is often possible in animal experiments where anorexia results in diminished food intake and the introduction of complicating deficiencies. After signs of deficiency had developed thiamin, riboflavin and brewer's yeast were added to the diet in series as shown in Figure 1 and their effects were studied. These studies dealt chiefly with carbohydrate metabolism and included the determination of the response of the organism to the administration of glucose as measured by blood sugar and respiratory quotient the response to a standard dose of insulin and

² The term vitamin B as used occasionally in this paper refers to the B complex as a whole. Individual members of the complex are designated separately.

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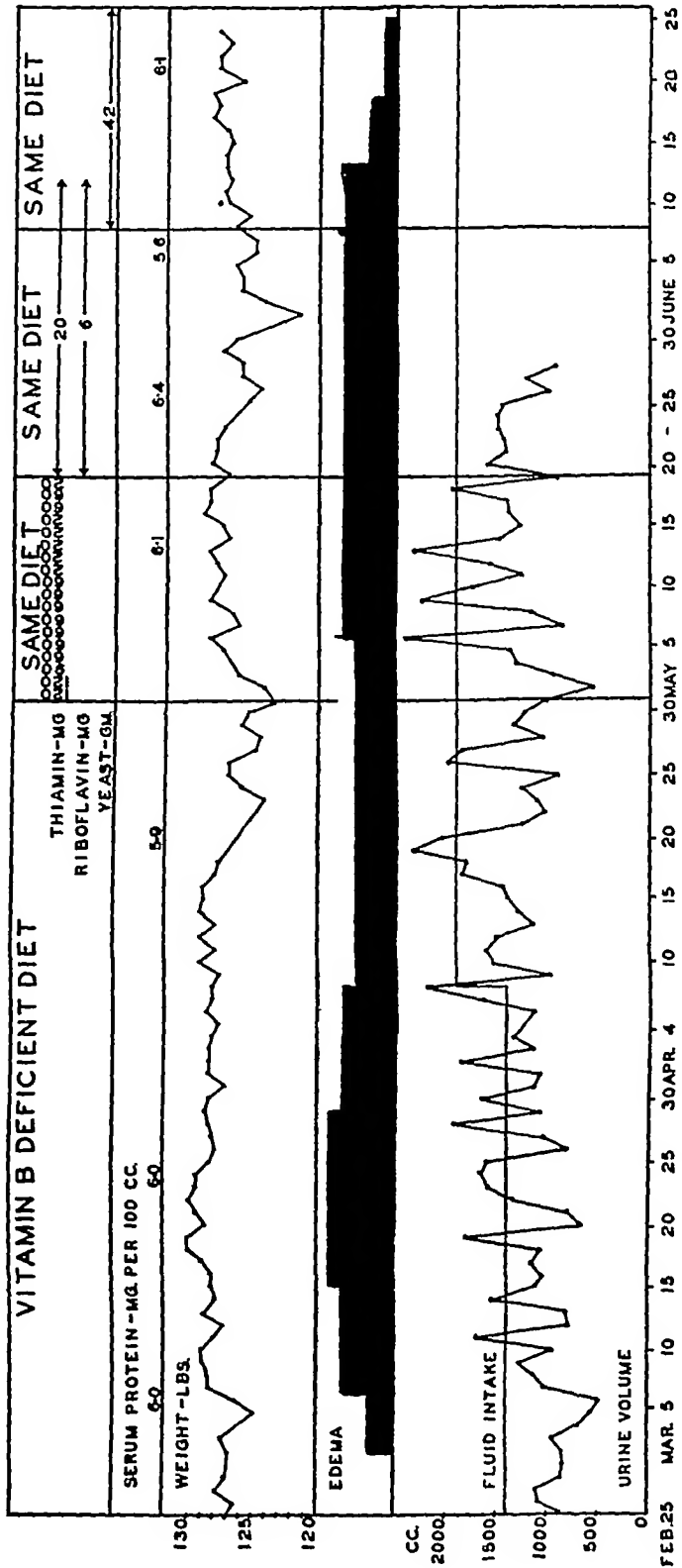


FIG 1 ALTERATIONS IN URINE VOLUME, FLUID INTAKE, EDEMA, BODY WEIGHT AND SERUM PROTEIN WHICH OCCURRED WHILE THE SUBJECT CONSUMED THE VITAMIN B DEFICIENT DIET AND FOLLOWING THE ADDITION TO THE DIET OF THIAMIN, RIBOFLAVIN, AND OF YEAST. Figures for urine volume were not obtained after May 28, due to the extreme temperatures which prevailed at that time.

estimations of blood pyruvic acid, lactic acid and bisulphite-binding substances before and after the ingestion of glucose. In addition, studies were made of serum protein and alteration in weight and fluid balance.

METHODS OF STUDY

The subject, a healthy woman aged 60 who had served in a previous study (14) of vitamin B deficiency volunteered to consume a diet deficient in the vitamin B complex. The observations were made in the metabolic ward of the University of Pennsylvania Hospital from February 25 to June 25, 1938, during which time she consumed daily the experimental diet outlined in Table I. The articles of diet, in the quantities indicated, supplied protein, fat, carbohydrate and total calories in optimal amounts. The vitamin B₁ content of the diet was about one-third of her calculated minimal requirement.

The vitamin B₁ of the diet is expressed in milligram equivalents *i.e.* the amount of vitamin B₁ in each food which is equivalent in terms of biological assay to a corresponding number of milligrams of a standard yeast powder. The theoretical minimal requirement of vitamin

B₁ of this individual was calculated from the formula of Cowgill (15)

$$VIT_1 = K_1 W_1 CAL_1$$

where VIT_1 is the vitamin (in milligram equivalents) required daily by an individual whose body weight, W_1 is expressed in grams. CAL_1 (kilogram calories) represents the total daily energy exchange, for which the calculated caloric value of the diet (2544 calories) has been used. K_1 is a species constant which, for man, Cowgill has determined to be 0.0000284. The vitamin B₁ requirement of this subject, whose initial weight was 58.5 kilograms, was found to be 4225 mgm. eq per day.

It was assumed that the amounts of other members of the B complex were proportionately decreased in the diet. Vitamins A, C and D, iron, calcium and sodium chloride in amounts thought to be optimal supplemented the diet as indicated in Table I. Except for days when experimental procedures required that food be withheld and for one 5-day period beginning April 18, when abdominal symptoms suggestive of appendicitis made a marked reduction of the diet advisable, the intake of food and food supplements was constant from the time of admission to discharge. The loss of weight during the period of low food intake (April 18-20) is explained by the decrease in the diet. Daily fluid intake, as made up by the liquid components of the diet, was 1400 cc. until April 8 when it was increased to 1900 cc. The term "fluid balance" as used in the text refers to the relation of fluid intake to urine volume.

The experimental regimen, divided into the 5 following consecutive periods, was begun immediately on admission to the hospital (1) the first week on the deficient diet, called "first period" in the tables which was regarded as a control period (2) the subsequent 8 weeks on the same diet, during which time signs of deficiency appeared (this period was terminated because of the development of clinical manifestations of deficiency demanding treatment)* (3) a period of 18 days during which thiamin hydrochloride alone was added to the deficient diet in doses ranging from 20 to 120 milligrams daily (4) 20 days during which riboflavin was administered, 6 milligrams daily in addition to thiamin (5) the final 18 days when brewer's yeast† was given, 42 grams daily. This period was shorter than desired due to unavoidable circumstances, so that the effects of this therapeutic agent were

* The clinical manifestations of deficiency which were observed, together with clinical studies made upon the subject during each experimental period, will be reported in a separate communication.

† This material (as Betabion) and the riboflavin were supplied through the courtesy of Mr P. C. Ackerman of Merck and Company.

‡ The brewer's yeast was kindly supplied by Dr J. H. Harris of the Harris Laboratories, Tuckahoe, New York, as "Brewer's Yeast Harris Medicinal Powder". Our analysis showed that its nitrogen content was 7.8 per cent. By the usual calculation ($N \times 6.25$) this supplied 21 grams of protein in addition to that of the diet.

TABLE I

Composition of the deficient diet and its supplements (per day)

Food	Weight grams	Protein grams	Fat grams	Carbo- hydrate grams	Calories	Vita- min B ₁ mgm.* equiv.
FOODS						
Cream of wheat (dry weight)	32	6		24	110	96
Skim milk	25	1		1	8	35
Blackut†	323†	21	39	146	1020	510
Butter	20		16		144	118
Jelly	72	3		48	219	
Lamb chop	20	5	4		56	200
Spinach	28	0.5		2	10	50
Rice (dry weight)	32	3		24	108	51
Letting	25	1			4	40
Baked apple caramel sauce	75	5	7	37	231	180
Cheese (American)	20	4	6		70	40
Gelatine	17	16.5		25	166	
Sugar	100			100	400	
Total		66	72	408	2544	1330
FOOD SUPPLEMENTS						
Calcium gluconate	3					
Sodium chloride	3					
Ferrous sulphate (Froese)	0.6					
Ascorbic acid (Cablone)	0.1					
Halibut liver oil with Vitamin	2 capsules					

* See text.

† Recipe for blackut

Flour	170 grams	Weight of recipe 323 grams.
Lard	38 grams	
Baking powder	2 tsp.	
Water	113 grams	

evident but probably not maximal before it was necessary for the subject to leave the hospital

The subject was weighed daily under standard conditions. Total output of urine was recorded daily. Total serum protein was determined occasionally. Fasting blood sugar determinations and sugar tolerance tests were carried out several times in each period. Venous blood sugar, as true blood sugar, was determined before and at hourly intervals for 4 hours after the ingestion of 100 grams of glucose. The blood sugar was determined by the micro method of Fohn and Malmros (16) modified for use with the Evelyn photoelectric colorimeter (17). Urine, collected before and at hourly intervals throughout the test, was analyzed qualitatively for sugar and ketones. The respiratory quotient was determined, using the Tissot apparatus, before each sugar tolerance test and hourly for 3 to 4 hours after the glucose had been given. Blood lactic acid was determined by the method of Friedemann and Graesser (18), pyruvic acid by the method of Wendel (19). Bisulphite-binding substances in the blood were determined by a slight modification of the method of Clift and Cook (20). These analyses were made upon the fasting subject at various times during observation and at 1, 2 and 4 hours following the administration of 100 grams of glucose. The response to insulin was studied under basal conditions at various times during each experimental period, 25 units of insulin being given subcutaneously in each test. Blood and urine samples were obtained prior to giving the insulin and at $\frac{1}{2}$, 1, 2 and 3 hours thereafter.

RESULTS

1 Alterations in weight and fluid balance

Changes in weight at first corresponded to alterations in fluid balance but late in deficiency the subject lost weight in spite of fluid retention (Figure 1). The 24-hour urinary nitrogen excretion was determined for 3 days of each experimental period. Table II shows these nitrogen values as well as the 24-hour urine volumes.

Edema appeared 5 days after the diet was begun. As in earlier studies of vitamin B deficiency (14) no adequate explanation for its occurrence is available. Alterations in serum protein were not sufficient to account for it. Previous attempts to demonstrate increased capillary permeability or increased capillary pressure yielded negative results, and were not repeated during this study. Periods of fluid retention and periods of fluid loss began and terminated suddenly without known change in the subject's environment. From the 10th to the 50th day of observation variations in edema and in fluid balance could account for the observed fluctuations in weight.

TABLE II
Daily urine volume and urine nitrogen during the experimental periods

Date 1938	Urine volume	Urine nitrogen	Period
	cc. per day	grams per day	
Feb 27	1102	4.5	First period
March 1	1105	4.4	
March 3	944	6.7	
April 27	1883	3.3	Vitamin B deficient diet (9th week)
April 28	1063	3.1	
April 29	1389	4.2	
May 17	1421	3.7	Deficient diet + thiamin
May 18	1916	3.0	
May 19	899	2.8	
June 3	828	5.1	Deficient diet + thiamin + riboflavin
June 4	1578	4.2	
June 5	1720	4.1	
June 17	1992	7.6	Deficient diet + yeast
June 18	1270	6.7	
June 21	774	7.1	

After 50 days on the diet, the weight declined coincident with the temporarily restricted diet. It rose when the full diet was resumed but did not return to the previous level and it fell again before thiamin was given. The weight was restored by thiamin. The sharp, temporary fall during the riboflavin period accompanied a fasting day on May 31. However, the maintenance of weight during the yeast period, at a time when edema was subsiding, suggests that yeast may contain some factor or factors, other than thiamin and riboflavin, which control the regulation of body fluid and perhaps the building of body tissues.

2 Blood sugar and respiratory quotient following glucose

The blood sugar concentration was determined at hourly intervals after the oral administration of glucose in each experimental period (Table III). During the first period and upon the initial examination in the period of deficiency the values are within the normal range for glucose tolerance curves. Likewise, the rise in blood sugar during the first and second hours following the administration of glucose was within normal limits throughout all periods of observation. However, beginning late in deficiency (April 18) and continuing until after 12 days of administra-

TABLE III

Blood sugar fasting and following the oral administration of 100 grams of glucose in the different experimental periods

Date 1938	Blood sugar					
	Fasting	1 hour	2 hours	3 hours	4 hours	
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	
March 1	92	174	145	126	85	First period
March 4	70	135	150	132	93	
March 23	63	108	127	88	80	
April 5	69	155	121	119		Deficient diet
April 18	61	124	116	138	108	
May 4	74	148	145	155	153	
May 10	80	152	159	143	122	Deficient diet + thiamin
May 23	85	145	146	124	137	
June 6	73	140	151	142	132	Deficient diet + thiamin + riboflavin
June 15	73	158	160	136	130	
June 20	65	139	115	101	89	

tion of yeast (June 20) the 4th hour blood sugar values failed to return to near the fasting value. These 4th hour sugars exceeded the fasting values by 47 to 79 milligrams per cent, while during the control period and following the administration of yeast the maximum elevation over the fasting value at 4 hours was 24 milligrams per cent. At the same time that the 4th hour values were elevated the 3rd hour values tended to exceed those obtained during the first period and after yeast. The urine at no time contained sugar or ketone bodies. Since the peak of the blood sugar curve after glucose was not significantly altered it was assumed that the absorption of glucose from the gastro-intestinal tract was not impaired. This conclusion was borne out also by a single measurement of the absorption of glucose from the small intestine during deficiency⁶ using the Miller Abbott tube (21) and employing a modification of the method of Groen (22) which gave a result within the normal range. The removal of glucose from the blood stream however appeared to be diminished as judged by the 3rd and 4th hour

blood sugar figures. This defect became definite late in deficiency was unaffected either by thiamin or riboflavin but disappeared following the administration of yeast. A reasonable explanation for these variations is that interference with glycogen deposition in the liver had occurred. Impaired glycogenesis in the liver following the parenteral administration of glucose has been demonstrated in animals deficient in the B complex (8, 9, 10). Whatever the mechanism may be, thiamin and riboflavin were without effect upon the glucose tolerance curve while yeast appeared to restore the ability to utilize carbohydrate normally.

It is of considerable interest that the findings relative to blood sugar observed in this subject during deficiency are comparable to the changes of early inanition (23) and also to the changes recently described in liver disease (24). In B₁ deficiency in animals hyperglycemia (7), glycosuria and the appearance of ketone bodies in the urine have been reported suggesting a diabetic like state. In human diabetes the administration of vitamin B₁ was thought to have resulted in an improved sugar tolerance (25). However nothing to suggest a diabetic tendency was observed in the present subject either during partial deficiency or following the administration of thiamin or riboflavin.

Throughout the periods of deficiency and therapy the basal metabolic rate ranged from -12 to +5, the average being -8 per cent.

Disturbances in the respiratory quotient following the administration of glucose to animals both during B₁ deficiency and after the administration of thiamin have been reported (11). Our findings (Table IV) show no significant variations in the fasting respiratory quotient, and the response of the quotient to glucose appeared normal at all times.

3. Blood bisulphite binding substances pyruvic and lactic acid

During the course of the experiment, weekly fasting values for total bisulphite-binding substances lactic acid and pyruvic acid were obtained on protein- and sugar-free filtrates. During the glucose tolerance tests these substances were determined also at intervals of approximately 1 or 2 and 4 hours after the ingestion of glucose.

⁶ We are indebted to Dr. Paul M. Glenn and Dr. Olive D. Hoffman for the performance of this experiment.

TABLE IV

Respiratory quotient, fasting and following the oral administration of 100 grams of glucose in the different experimental periods

Date 1938	Fasting	1 hour	2 hours	3 hours	4 hours	
March 1		0 90	0 94	0 98	0 97	First period
March 4	0 75	0 83	0 95	0 98	0 98	
March 23	0 91	0 89	0 96	0 94	0 93	
April 5	0 87	1 00	0 97	0 99		Deficient diet
April 18	0 79	0 92	0 88	0 92		
May 4	0 78	0 94	0 91	0 97		Deficient diet + thiamin
May 10	0 78	0 89	0 94	0 90		
May 23	0 88	0 95	0 91	0 94	0 89	Deficient diet + thiamin + riboflavin
June 6	0 81	0 94	0 91	0 92	0 98	
June 15	0 83	0 88	0 94	0 92	0 92	Deficient diet + yeast
June 20	0 88	0 96	0 96		0 95	

Table V gives the values obtained. The fasting bisulphite-binding power did not rise during the period of vitamin B deficiency nor were the values significantly elevated after glucose. Taylor, Weiss and Wilkins (26), as well as Platt and Lu (27), obtained elevated values for bisulphite-binding substances in fasting subjects with clinical vitamin B deficiency. Two explanations for the difference between our results and theirs may be considered: either that in our subject the deficiency did not proceed to such an advanced stage as to give this change, or that the clinical deficiencies studied were complicated by inanition or by deficiencies other than vitamin B. Inanition has been shown by Lipschutz and co-workers (28) to be responsible for a large part of the disturbances noted in the carbohydrate metabolism of vitamin B-deficient animals.

Pyruvic acid concentrations in the fasting blood of the subject were not increased during the period of vitamin B deficiency. However, after

TABLE V

Bisulphite-binding substances, lactic acid and pyruvic acid, fasting and following the oral administration of 100 grams of glucose in the different experimental periods

Date	BBS*			Lactic acid			Pyruvic acid			
	Fasting	1 hour after glucose	4 hours after glucose	Fasting	1 hour after glucose	4 hours after glucose	Fasting	1 hour after glucose	4 hours after glucose	
	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	
March 1	5 9	4 3	4 1	12 3	17 7	8 4	1 7	4 1	4 6	First period
March 8	4 0			17 6			4 3			
March 15	4 1			17 3			3 2			
March 22	4 5	4 7	4 8	27 1	17 6	13 2	4 6	5 9	0 6	Deficient diet
April 12	4 1	5 2	5 4	12 9	24 5†	13 8	2 3	6 6	8 3	
April 18	5 6	8 2	8 5	10 1	21 2	19 8	4 1	11 6	8 1	
April 22	2 9			20 9			2 1			
May 4	3 6	5 0	2 5	15 7	22 5	19 8	1 3	5 1	1 4	Deficient diet + thiamin
May 10	4 1	3 8	3 2	6 2	16 9	9 3	3 0	7 6	3 4	
May 24	2 3	4 9	1 8	7 0	18 9	7 5	0 1	6 8	1 5	Deficient diet + thiamin + riboflavin
May 31	3 1			5 6			2 7			
June 20		3 0	2 4		15 5	10 0		6 8	3 8	Deficient diet + yeast
June 23	4 8			11 5			0			

* BBS = Bisulphite binding substances.

† Two hours after glucose

glucose ingestion there was a rise in pyruvic acid, the values tending to remain elevated throughout the 4 hours of the test. Following the administration of thiamin, these 4th hour values for pyruvic acid returned to within the normal fasting range.

Pyruvic acid as determined by reduction to lactic acid, was always less than the total bisulphite-binding substances in the fasting blood of our subject, but it significantly exceeded bisulphite-binding substances after glucose ingestion. This discrepancy also occurred in 2 normal subjects and appeared greater than could readily be accounted for by the loss of some pyruvic acid from the filtrate before determination of bisulphite-binding power. Pyruvic acid contains a free carbonyl group which will quantitatively bind bisulphite. Since bisulphite binding power did not rise concurrently with pyruvic acid there may be errors in one or both determinations by these methods.

The values for lactic acid of the blood appear to be elevated during deficiency both before and after glucose, particularly as compared with values obtained following the administration of thiamin.

4 Response to insulin

Alteration in the response to insulin occurred during the experiment. As deficiency progressed, the subject became resistant to insulin. Following the administration of thiamin the response to the same dose of insulin was increased and when riboflavin was added insulin sensitivity developed (Table VI).

The 3 examinations made during the deficient period showed a progressive decrease, both in the percentage fall in blood sugar from the fasting level and in the symptomatic response to insulin. In the last test made during deficiency no fall in blood sugar occurred and the subject experienced no unusual symptoms. One week later, however large doses of thiamin having been administered (Figure 1) a 36 per cent fall in blood sugar occurred and the subject experienced moderate symptoms of insulin shock after the test dose. During the remainder of the thiamin period the response to insulin appeared to vary roughly according to the amount of thiamin administered immediately prior to the test. When, however riboflavin was added to a small dose of thiamin the

TABLE VI

Blood sugar before and following the subcutaneous administration of 2.5 units of insulin in the different experimental periods

Date 1938	Blood sugar					Maximal decrease in blood sugar*	
	Initial	1 hour	2 hours	3 hours	4 hours		
	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	per cent	
March 11	71	66	56	59	61	21	Deficient diet
March 25	70	70	63	68	78	10	
April 29	66	76	66	70	76	0	
May 6	77	53	49	56	62	36	Deficient diet + thiamin
May 13	66	64	65	65	66	3	
May 17	68	61	59	58		15	
May 27	86	58	44	52	56	49	Deficient diet + thiamin + riboflavin
May 31	131	101	67	48	62	63	

* Maximal decrease in blood sugar is here represented as the per cent fall from the initial blood sugar to the lowest value obtained in the test.

response was greater than that which had been obtained with thiamin alone. On May 27, when the subject was receiving daily 20 milligrams of thiamin and 6 milligrams of riboflavin she experienced definite symptoms of shock following the standard dose of insulin, at which time a fall of 49 per cent in blood sugar occurred. These symptoms were transient and required no treatment but on May 31 the test being repeated under identical conditions the blood sugar fell 63 per cent and symptoms of reaction became severe, in spite of the high initial blood sugar.

Altered response to insulin has been reported in animals deficient in the B complex, but the type of response appears to vary with the animal concerned. Rats deficient in the B complex (12) are reported sensitive to insulin while pancreatized tomized dogs maintained on a B-deficient diet became resistant to insulin (29). Little assistance is obtained therefore from these animal experiments in interpretation of the changes observed in the human subject.

SUMMARY

1 An otherwise normal individual, subsisting on a diet adequate except for the vitamin B complex, developed clinical manifestations and metabolic changes which responded in part to thiamin, were influenced only slightly by thiamin plus riboflavin and were relieved by the administration of brewer's yeast

2 In general, both clinical manifestations and metabolic changes were evident after 5 weeks on the experimental diet but did not become striking until the subject had consumed the deficient diet for 8 weeks

3 Observations are presented showing the changes in fluid balance and body weight, in nitrogen excretion, in carbohydrate metabolism, and in the response to insulin

4 Edema appeared early in deficiency, loss of body weight occurred late. Edema disappeared and normal body weight was maintained only following the administration of yeast

5 The changes in carbohydrate metabolism included

(a) Failure of the blood sugar to return to normal within 3 to 4 hours after the ingestion of glucose, this defect disappearing only after the administration of yeast. There was no evidence of failure to oxidize glucose as indicated by the respiratory quotient or of failure of absorption of glucose from the gastro-intestinal tract

(b) There was no increase in the bisulphite-binding power or in the fasting pyruvic acid of the blood during deficiency. After the ingestion of glucose the pyruvic acid rose and remained high at 4 hours until after the administration of thiamin. Blood lactic acid was elevated during deficiency and returned to normal following the administration of thiamin

(c) The respiratory quotient was unaltered during deficiency

(d) The response to insulin decreased as deficiency progressed. There was an increased response to insulin following the administration of thiamin and, with the addition of riboflavin, the subject became sensitive to insulin

also to Dr Herbert Fox in whose laboratory determinations of serum protein were carried out, and to Dr L. G Wesson who made several of the analyses for respiratory quotient.

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STUDIES ON THE ACTION OF SULFAPYRIDINE ON PNEUMOCOCCI

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In previous studies (1) it was shown that sulfanilamide in the concentrations ordinarily attained in therapy, exerts a marked bacteriostatic action on Type III pneumococcus when added to human blood *in vitro* or when found in the blood of patients with pneumonia after treatment. There was no appreciable bactericidal action except in some instances when large concentrations of the drug were used. This action was not associated with increased phagocytosis. Type-specific antibodies, whether actively acquired or when administered as therapeutic rabbit serum induced pneumococidal activity in the blood of the patients and this was accompanied by increased phagocytosis. The combination of anti serum and sulfanilamide, when added *in vitro* or when used therapeutically resulted in a greater bacteriostatic and bactericidal action of the blood than when either was used alone.

In the present paper the results of similar studies with sulfapyridine are reported. The action of this drug on Types I, II and V pneumococci was studied in the blood of non pneumonic subjects lacking pneumococcal properties and in blood taken before treatment from patients with pneumonia due to the homologous pneumococcus types. In the first portion of this paper the results of studies of the effect of sulfapyridine on the growth curve of pneumococci in artificial media and in human blood are also reported. The immune reactions of the blood of patients with pneumococcus pneumonia before and after treatment with sulfapyridine are reported separately (2)

The action of sulfapyridine on pneumococci *in vitro* has been studied by various techniques with somewhat conflicting results (3 to 7). McIntosh and Whitby (3) found active multiplication of pneumococci during 6 hours followed by marked bactericidal activity in 24- and 48-hour experiments. The bactericidal activity varied with the size of the inoculum and the concentration of the drug. Fleming using a "slide-cell" technique with whole and de leukocytized blood, found only bacteriostasis

as evidenced by smaller colony size after 24 hours in sulfapyridine-containing blood and as compared with controls. He was able to show bactericidal action only in the presence of leukocytes and this bactericidal action was enhanced by the addition of specific immune serum. Long Bliss and Feinstein (5) found sulfapyridine to be somewhat more effective than sulfanilamide as a bacteriostatic agent against Types I, II, and III pneumococci in broth cultures. Reid (6) using Type I pneumococci found slight bacteriostasis during the logarithmic phase of growth but incomplete bactericidal action later.

Hoyt and Levine (7) using large inocula of Type II pneumococci, found only bacteriostasis in serum and in peptone free broth in 8 hours' growth. In their experiment the effect of sulfapyridine was largely lost when peptone was added to a concentration of 2 per cent. McIntosh and Whitby (3) did not find this inhibiting effect of peptone in the action of sulfapyridine on Type I pneumococci. Sulfapyridine was found to have no effect on the immune mechanism in mice. Marked variations have been noted in the sensitiveness of various types and strains of pneumococci to the action of sulfapyridine *in vitro* and in experimental infections (8, 9). Resistance, or "fastness" has been artificially induced by growth in sulfapyridine and this fastness is then found to be independent of virulence and of capsule formation (9, 10).

MATERIALS AND METHODS

The blood used for the pneumococcal tests was obtained (1) from normal laboratory workers and hospital patients without recent febrile illness and (2) from patients with pneumococcus pneumonia before any serum or chemotherapy was started. Only such bloods were employed as lacked pneumococcal activity or other type-specific antibodies (opsonins, agglutinins and mouse protection) for the types of pneumococci used in the tests. Only homologous types were used in the blood of the pneumonia patients.

The pneumococcal and phagocytic tests were carried out as in previous studies (1) but with some modifications. The frequency with which considerable numbers of pneumococci were killed off at 48 hours in tubes which did not exhibit full growth led us to record the number of viable organisms in the tubes showing incomplete growth (that is, the number of colonies in plates poured at 48 hours).

Three hour test. While these studies were under way it became apparent that the observation of growth in inhibition and pneumococcal action after 48 hours as previously carried out, did not distinguish between the

killing power of the blood itself and that due to the action of the chemical. This could be accomplished, in part, by control tests with inactivated serum and/or "deleukocytized" blood, since either of these conditions interferes with the destruction of pneumococci in the absence of the drug. However, a simpler method was evolved. It was found, by serial colony counts made at frequent intervals, that the pneumococcal action residing in the blood is most marked during the first few hours of incubation while, as shown by McIntosh and Whitby (3), the action of sulfapyridine becomes apparent only between 2 and 8 hours and sometimes even later. In our own studies, this lag in the action of sulfapyridine lasted at least 4 hours. It was obvious, therefore, that colony counts done early in the test will not only differentiate the two individual activities, but the differences between these and the later ones will reflect more accurately the number of organisms killed through the action of the drug. After a number of experiments in which the inocula and the period of growth were varied, it was found most advantageous to pour plates after 3 hours' incubation from the tube containing blood in which 10^3 and 10^5 pneumococci had been inoculated. In following the course of patients under treatment, this test was of particular value in differentiating between the pneumococcal activity acquired through the development of immunity and that due to action of the drug. This will be shown in the studies carried out with patients' blood (2).

The pneumococci used in most of the experiments were the stock Types I, III, and V strains in use in this laboratory for a number of years. Type I and Type III strains were originally obtained from the Rockefeller Institute and the Type V strain from the late Georgia Cooper. Their virulence has been maintained by frequent mouse passage. They were grown in rabbit blood broth (beef infusion broth with 1 per cent peptone and 0.05 per cent dextrose, pH 7.8, to which 1 per cent defibrinated rabbit blood is added). All pour plates were made in beef infusion agar, pH 7.8, to which 5 per cent horse blood was added. The pneumococcal tests were usually done with 3- to 4-hour cultures made with a 50 per cent inoculum, while in the growth curves the cultures used were in the stationary growth phase after 10 hours' growth from a small inoculum. In either case, pour plates of the cultures used yielded between 45 and 120 colonies from 10^{-7} cc. For the stock strains, unless otherwise noted, 0.1 cc. of this dilution of culture is referred to as having 10 pneumococci. In the case of strains freshly isolated from patients, cultures grew more slowly and more sparsely (10^{-7} cc. usually yield 10 to 20 colonies).

Growth curves were studied in blood broth and in whole defibrinated blood which lacked pneumococcal action. When blood was prepared for such tests, the proper amounts of the blood, the culture dilution, and sulfapyridine were introduced into a single tube in proportions corresponding to those used in the pneumococcal tests. The mixture was then distributed in 0.5 cc amounts in a number of pyrex tubes which were then sealed in an oxygen flame, placed in the incubator and

rotated. The tubes were broken open individually at intervals and agar plates poured either with the entire contents or with serial dilutions.

Growth curves in broth were carried out by adding 0.2 cc. of a 10-hour culture to 18 cc. of the blood broth with or without sulfapyridine and serial decimal dilutions were made in the same medium. The various tubes were then incubated and 0.1 cc amounts were removed at various intervals for culture in agar pour plates, either directly or after proper dilution. Complete bactericidal activity was assumed whenever 0.1 cc of the material yielded no growth. However, in some instances when blood was used, the entire contents of the tube were cultured in rabbit blood broth at the conclusion of the experiment in order to ascertain whether bactericidal action was complete.

Sulfanilamide and sulfapyridine determinations were made by the method of Marshall and Litchfield (11).

The therapeutic serums used were chiefly concentrated horse and rabbit serums furnished for clinical trial by the Lederle Laboratories.

RESULTS

Effect of sulfapyridine on the growth curve of the pneumococcus

Stock Type III pneumococcus in blood broth
Control growth curves for this organism are shown in Figure 1. Practically no lag phase was noted, even with small inocula. This absence of lag phase, as will be seen in the figures which follow, was characteristic of the growth of all the strains both in this media and in blood, and occurred with all inocula, irrespective of whether or not sulfapyridine was present (at least in the concentrations used).¹ The maximum growth without sulfapyridine was attained in about 12 hours with the smaller inocula and as early as 4 hours with the larger inocula. There was a slow decline in population after a stationary phase of 12 to 24 hours.

The effect of sulfapyridine in concentrations of 5 to 10 mgm per 100 cc. of the media, is shown in Figures 2 and 3, respectively. Rapid multiplication occurred within the first 4 hours in every instance, even with an initial population of 10 organisms and with a sulfapyridine concentration of 10 mgm per 100 cc. The rate of growth in sulfapyridine was about the same as without the drug in this interval. Between 4 and 8 hours

¹ This is not strictly true since a more detailed study during the first 4 hours indicates a lag of growth for $1\frac{1}{2}$ to 2 hours both with and without sulfapyridine.

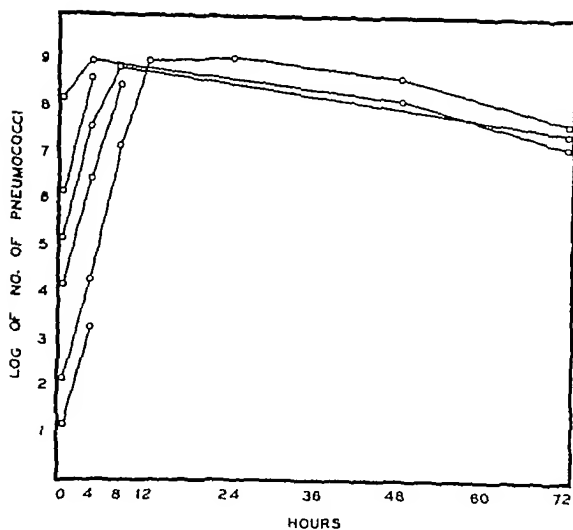


FIG. 1 GROWTH CURVES OF STOCK TYPE III PNEUMOCOCCUS IN BROTH WITHOUT SULFAPYRIDINE

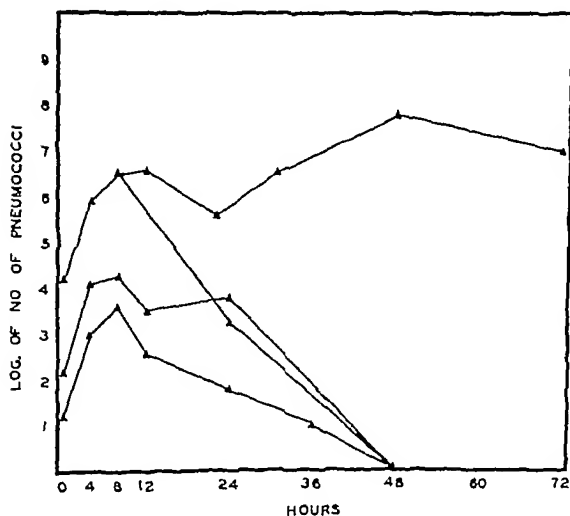


FIG. 2 GROWTH CURVES OF STOCK TYPE III PNEUMOCOCCUS IN BROTH CONTAINING SULFAPYRIDINE, 5 MG. PER 100 CC.

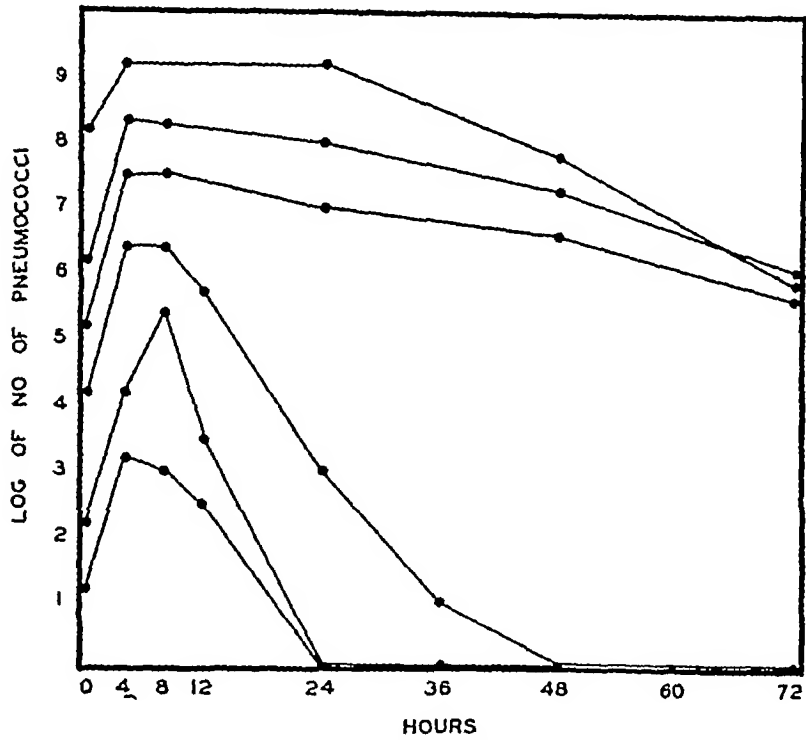


FIG. 3 GROWTH CURVES OF STOCK TYPE III PNEUMOCOCCUS IN BROTH CONTAINING SULFAPYRIDINE, 10 MG. PER 100 CC.

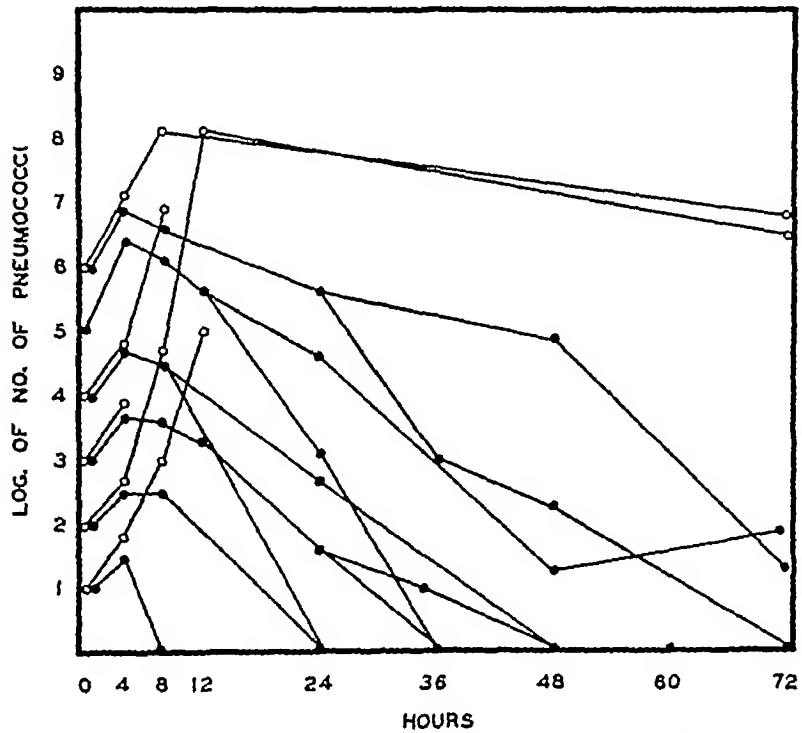


FIG. 4 GROWTH CURVES OF TYPE III PNEUMOCOCCUS, STRAIN "COUGHLIN" IN BROTH

○ = no sulfapyridine
● = sulfapyridine, 10 mgm. per 100 cc

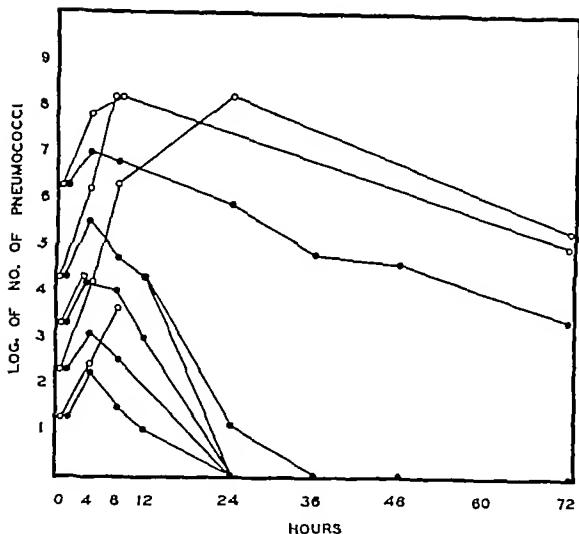


FIG. 5 GROWTH CURVES OF TYPE III PNEUMOCOCCUS STRAIN "GILLYARD" IN BROTH

○ = no sulfapyridine

● = sulfapyridine, 10 mgm. per 100 cc.

there was a stationary phase. With a sulfapyridine concentration of 5 mgm. per cent and an initial population of 10^8 pneumococci or more, growth occurred at about the same rate as in the controls. With smaller inocula in this concentration of the drug there was a steady decline in the number of viable organisms after the stationary period, and none could be grown after 48 hours. In one experiment, slow growth continued up to 48 hours with an inoculum of 10^4 organisms (Figure 2). With a sulfapyridine concentration of 10 mgm per cent, there was a more rapid decline in the number of viable organisms so that none could be recovered at the end of 24 hours when 10 or 100 organisms were inoculated. With an original inoculum of 10^4 diplococci there were less than 10 viable organisms per cc. after 36 hours, and none after 48 hours.

Growth curves of 3 different strains of Type III pneumococcus were studied in rabbit blood broth with and without 10 mgm of sulfapyridine per 100 cc. Strain "Coughlin" (Figure 4) was

obtained from a blood culture, and strains "Gillyard" (Figure 5) and "Taylor" (Figure 6) were isolated from sputum. Each strain was obtained before treatment with sulfapyridine was begun. All 3 patients recovered after treatment although the first had a very protracted illness. Taylor had a mild atypical pneumonia, but 1 year previously he had had a severe attack of Type III pneumococcal lobar pneumonia from which he had made a rapid recovery following specific serum therapy.

There were some differences noted in the growth curve of each of these 3 strains and, in some instances considerable variations were noted when the same tests were repeated. Some of the latter variations are indicated in the figures. In general, these 3 strains grew somewhat more slowly and the maximum growth showed considerably fewer viable organisms than in the case of the stock Type III strain. All exhibited rapid growth during the first 4 hours, irrespective of the size of the inoculum. In the media containing

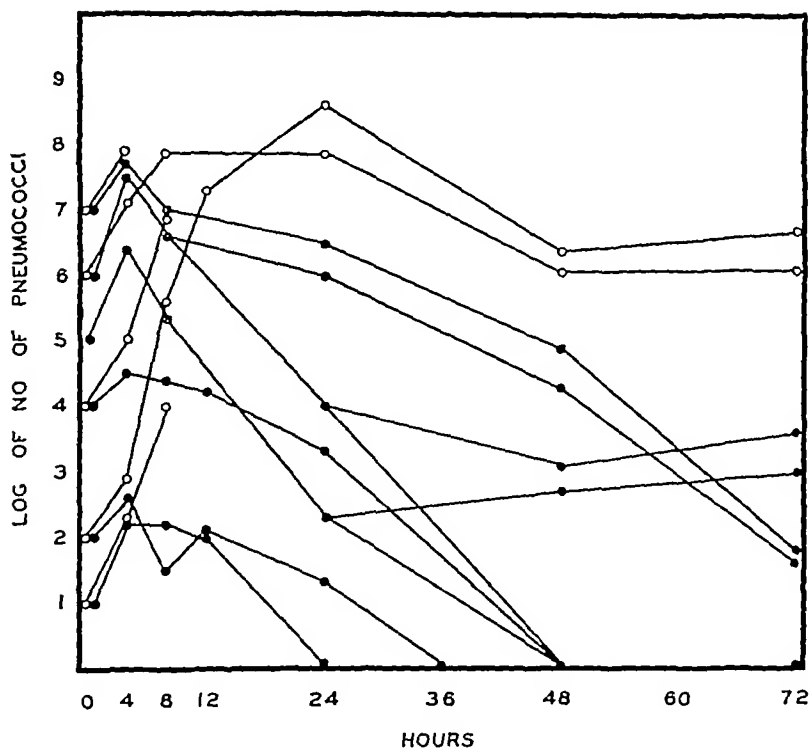


FIG 6 GROWTH CURVES OF TYPE III PNEUMOCOCCUS, STRAIN "TAYLOR"
IN BROTH

○ = no sulfapyridine
● = sulfapyridine, 10 mgm. per 100 cc

10 mgm of sulfapyridine per 100 cc, killing was complete with inocula of 10^4 pneumococci or less in the case of the Coughlin and Gillyard strains. Incubation for 36 to 48 hours was necessary to accomplish this in some of the tests which started with 10^3 and 10^4 diplococci. With larger inocula there was growth inhibition with a slower decline in the number of viable organisms after 4 to 8 hours.

Stock Type I and Type V strains The growth curves for these organisms in blood broth media with and without sulfapyridine 10 mgm per 100 cc are shown in Figures 7 and 8, respectively. For the most part, the curves for both these strains were similar to those obtained with the stock Type III pneumococcus. The decline in the number of viable organisms began after 4 hours' growth with most inocula up to 10^8 organisms in the case of the Type I pneumococcus, whereas with the Type V strain this decline apparently began after 8 hours.

Stock Type III strain in defibrinated human blood The blood used was from a patient without infection. Repeated tests showed free growth of Type III pneumococci in this blood even with the smallest inocula. Growth curves were carried out several times without sulfapyridine and with 5 and 10 mgm per cent of this drug. The control curves were all similar to those already shown for this organism in rabbit blood broth, except that a greater final population was attained in the blood. The curves with sulfapyridine 10 mgm per cent and small original inocula all showed the typical rise and rapid fall, killing being complete in 24 hours. With the drug in a concentration of 5 mgm per cent, growth occurred in varying degrees with an inoculum of 10 diplococci per cc, after which the number of viable organisms remained more or less static for 48 hours or more. A few representative curves are shown in Figure 9.

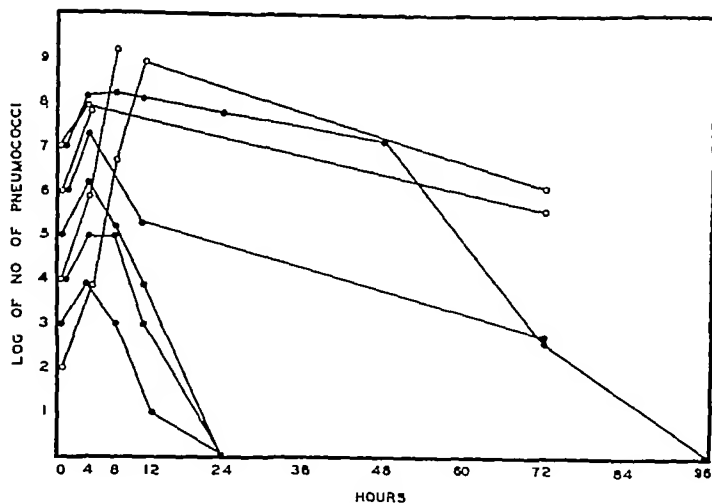


FIG. 7. GROWTH CURVES OF STOCK TYPE I PNEUMOCOCCUS IN BROTH

○ = no sulfapyridine

● = sulfapyridine, 10 mgm. per 100 cc.

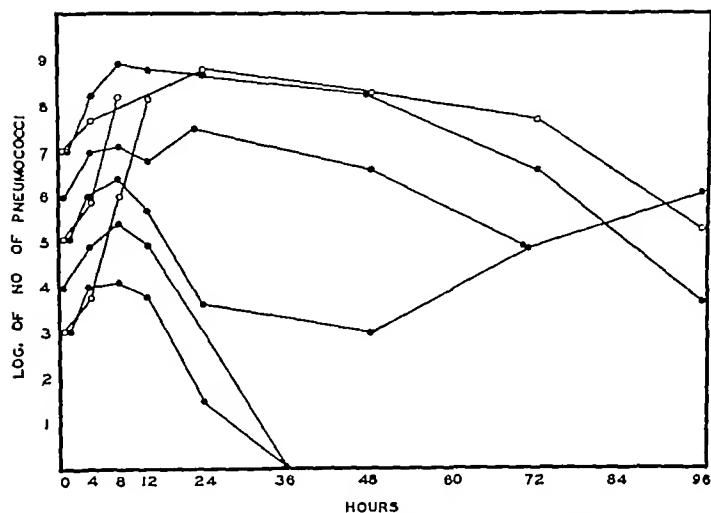


FIG. 8. GROWTH CURVES OF STOCK TYPE V PNEUMOCOCCUS IN BROTH

○ = no sulfapyridine

● = sulfapyridine, 10 mgm. per 100 cc.

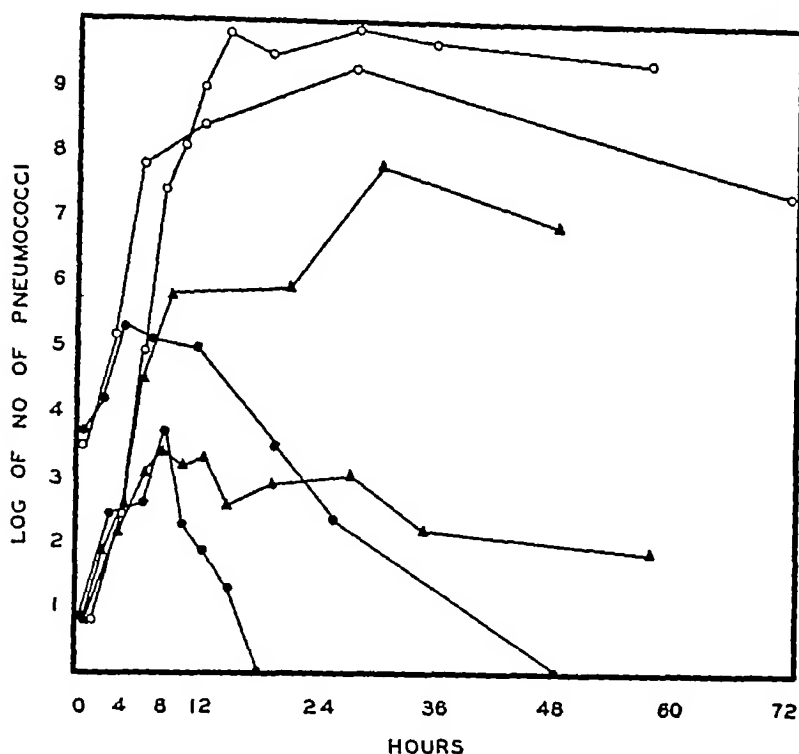


FIG. 9 GROWTH CURVES OF STOCK TYPE III PNEUMOCOCCUS IN DEFIBRINATED HUMAN BLOOD

○ = no sulfapyridine
 ▲ = sulfapyridine 5 mgm. per 100 cc.
 ● = sulfapyridine 10 mgm. per 100 cc.

Effect of certain environmental factors on the growth curve of Type III pneumococcus

Effect of exposure to a temperature of 5° C
 Blood broth cultures were inoculated with various numbers of pneumococci and exposed to a temperature of about 5° C for periods of 4 to 72 hours either immediately after inoculation or after 4 hours' growth. Further incubation at 37° C was resumed in most instances after this exposure to cold. The resulting growth curves with and without sulfapyridine are shown in Figures 10 and 11, respectively. The exposure to 5° C resulted in a fairly static population for the duration of this exposure. After reincubation at 37° C, the resulting growth curve was essentially the same as though no exposure to cold had taken place.

Growth at 27° C Growth curves carried out at this temperature with and without sulfapyridine 10 mgm per cent showed a lag phase of several

hours' duration. The subsequent multiplication was considerably slower than at 37° C. The effect of sulfapyridine, however, was the same as at 37° C. Bacteriostasis occurred with the large inocula and killing was complete when a small inoculum was used. There was a longer stationary phase after multiplication occurred, but the rate of decline in population was rapid in the cultures that started with a small inoculum.

Growth at 40° C The stock Type III strain used is not a heat labile strain (12) and growth without sulfapyridine was essentially the same at approximately 40° C as at 37° C during a 48-hour period. With sulfapyridine 10 mgm per cent, the effect was the same as at 37° C during the first 48 hours. After that time there was a decline in population in the control cultures, but a more rapid decline occurred in those containing sulfapyridine.

Effect of 2 per cent peptone The media used throughout contained 1 per cent peptone. The

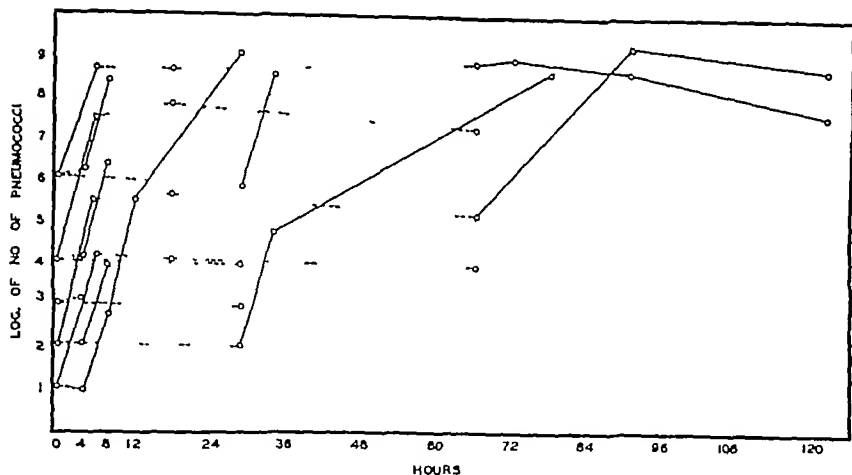


FIG 10 GROWTH CURVE OF STOCK TYPE III PNEUMOCOCCUS IN BROTH WITHOUT SULFAPYRIDINE

Solid line = growth at 37° C.

Broken line = in ice box at 5° C.

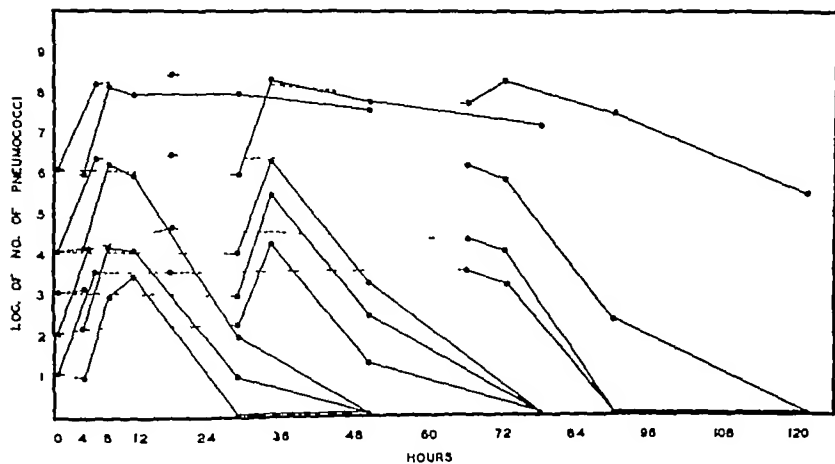


FIG 11 GROWTH CURVE OF STOCK TYPE III PNEUMOCOCCUS IN BROTH WITH SULFAPYRIDINE 10 MG. PER 100 CC.

Solid line = growth at 37° C.

Broken line = in ice box at 5° C.

TABLE I

The in vitro action of sulfanilamide, sulfapyridine, and homologous immune rabbit serum on pneumococci in fresh defibrinated blood lacking pneumococidal activity

A RESULTS OF TESTS ON THE BLOOD OF NON-PNEUMONIC SUBJECTS										B RESULTS OF TESTS WITH HOMOLOGOUS PNEUMOCOCCUS TYPE IN BLOOD TAKEN BEFORE TREATMENT FROM PATIENTS WITH PNEUMONIA									
Added to 0.5 cc. blood†			Pneumococidal action at 3 hours		Growth inhibition		Pneumococidal action at 48 hours		Phagocytic index	Added to 0.5 cc. blood†			Pneumococidal action at 3 hours		Growth inhibition		Pneumococidal action at 48 hours		Phagocytic index
Sulfa-nil-amide	Sulfa-pyri-dine	Serum	In-oculum	Growth	24 hours	48 hours	In-oculum	Growth		Sulfa-nil-amide	Sulfa-pyri-dine	Serum	In-oculum	Growth	24 hours	48 hours	In-oculum	Growth	
mgm. per 100 cc.	mgm. per 100 cc.	units								mgm. per 100 cc.	mgm. per 100 cc.	units							
Pneumococcus Type III—3 Subjects										Pneumococcus Type III—13 Patients									
0	0	0	10 ³	88	0	0				0	0	0	10 ²	400-2000	0	0		750	0.9
5	0	0	10 ²	156	10 ² -10 ³	0				20	0	0	—	—	10 ²	10 ²	10 ²	750	0.5
10	0	0	10 ²	200	10 ⁴	10 ² -0	10	∞		0	2.5	0	10 ²	1200	10 ⁴	10 ² -10	10 ²	1180	
15	0	0	—	—	10 ⁴	0				0	3.75	0	10 ²	400	10 ²	10 ⁴	10 ²	∞	
20	0	0	10 ²	200	10 ⁴	10 ² -10 ²	10	0(2), ∞		0	0	0	—	—	10 ⁴	10 ⁴	10 ²	160 300	
								10 ²		0	5.0	0	—	—	10 ⁴	10 ⁴	10 ²	600	
0	2.5	0	10 ²	240	10 ⁴	10 ²	10 ²	10 ²		0	7.5	0	10 ²	280-1200	10 ²	10 ² -10 ⁴	10 ²	3	
0	5.0	0	10 ²	240	10 ⁴	10 ⁴	10 ²	0(2) ∞		0			—	—			10 ⁴	320	
								10 ²					—	—			10 ⁴	0(4) 2, 9	
0	7.5	0	10 ²	240	10 ⁴	10 ²	10 ²	90, 625, ∞					—	—			10 ⁴	0(3) 4, 7 70	0.0
								10 ²					—	—			10 ⁴	0 34, 92 180	
0	10	0	10 ²	140	10 ⁴	10 ²	10 ²	0(2) 103					—	—			10 ⁴	280 300, 350	
								10 ⁴					—	—			10 ⁴	40, 110, 1000 ∞(3)	
0								0, 15, 1500					—	—			10 ⁴	0(2) 40	
0	15	0	10 ²	50	10 ⁴	10 ²	10 ²	0, 15, 400, ∞		0	10	0	—	—	10 ²	10 ⁴	10 ⁴	0 10 61	
0	0	0.02	10 ²	1600	10 ²	10 ²	10 ²	0, 165		0	0	0.1	10 ²	4	10 ²	10 ²	10 ⁴	250 500	
0	0	0.2	10 ²	6	10 ²	10 ²	10 ²	0		0	0	2.0	10 ²	28	10 ²	10 ²	10 ⁴	0	0.7
0	0	2.0	10 ²	37	10 ²	10 ²	10 ²	0	+	0	0	20.0	—	—	10	10	10 ²	0	0.5
0	2.5	0.2	10 ²	17	10 ²	10 ²	10 ²	0		0	2.5	2.0	10 ²	40	10 ²	10 ²	10 ⁴	800	+
										0	7.5	4.0	—	—	10 ²	10 ²	10 ²	2	
										0	10	20	—	—	10 ²	10 ²	10 ²	23	
													—	—	10 ²	10 ²	10 ²	7	1.9
Pneumococcus Type I—2 Subjects										Pneumococcus Type I—6 Patients									
0	0	0	10 ²	1200	0	0				0	0	0	10 ²	200-80	0	0			1.0
5	0	0	10 ²	400	0	0							—	—	10 ²	10 ²	10 ²	90	1.7
10	0	0	10 ²	400	10 ² -10 ²	0				20	0	0	—	—	10 ²	10 ²	10 ²	540	2.0
15	0	0	10 ²	125	10 ²	10 ²	10	∞		0	3.75	0	—	—	10 ²	10 ²	10 ²	0	
20	0	0	10 ²	150-280	10 ² -10 ⁴	10 ² -10 ²	10	0 3000		0	5.0	0	10 ²	20-24	10 ²	10 ²	10 ²	0 4000	1.0
								10 ²					—	—			10 ²	36, ∞	
0	2.5	0	10 ²	380	10 ⁴	0	10 ²	0 85		0	7.5	0	—	—	10 ²	10 ²	10 ²	42 ∞	
0	5.0	0	10 ²	240	10 ² -10 ⁴	10 ² -10 ²	10 ²	290, 2000		0	10	0	10 ²	40	10 ²	10 ²	10 ²	0 2000	
								10 ²					—	—	10 ²	10 ²	10 ²	0 85	
0	7.5	0	10 ²	200	10 ²	10 ²	10 ²	8		0	15	0	10 ²	35	10 ²	10 ²	10 ²	25, 400	
								10 ²		0	0	0.3	10 ²	2	10 ²	10 ²	10 ²	0	0.8
0	10	0	10 ²	90	10 ²	10 ²	10 ²	5000		0	0	2.5H	—	—	10 ²	10 ²	10 ²	0	
								10 ²		0	0	5.0	—	—	10 ²	10 ²	10 ²	0	
0								10 ²		0	0	10.0	—	—	10 ²	10 ²	10 ²	0	10.0
0	15	0	10 ²	26	10 ²	10 ²	10 ²	0 15		0	0	25.0H	—	—	10 ²	10 ²	10 ²	0	11.1
								10 ²		0	0	—	—	—	10 ²	10 ²	10 ²	0	
0	0	0.03	10 ²	4	10 ²	10 ²	10 ²	0 ∞		0	3.75	5.0	—	—	10 ²	10 ²	10 ²	0	13.9
0	0	0.06	10 ²	3	10 ²	10 ²	10 ²	0		0	5.0	0.3	10 ²	2	10 ²	10 ²	10 ²	0	0.9
0	0	0.12	10 ²	6	10 ²	10 ²	10 ²	0		0	5.0	8.0	10 ²	0	10 ²	10 ²	10 ²	0	14.9
0	0	0.3	10 ²	4	10 ²	10 ²	10 ²	0					—	—	10 ²	10 ²	10 ²	13	
									0.5				—	—	10 ²	10 ²	10 ²	0	
0	0	0.5	10 ²	0	10 ²	10 ²	10 ²	0		0	10	2.54	—	—	10 ²	10 ²	10 ²	0	
0	0	1.0	10 ²	3	10 ²	10 ²	10 ²	0					—	—					
0	0	3.0	10 ²	9	10 ²	10 ²	10 ²	0					—	—					
0	0	30.0	10 ²	68	10 ²	10 ²	10 ²	0	20.1				—	—					
0	0	0.03	10 ²	75	10 ²	10 ²	10 ²	0	11.4				—	—					
5	0	3.0	—	—	10 ²	10 ²	10 ²	0	25.5				—	—					
5	0	0.03	10 ²	280	10 ²	10 ²	10 ²	0					—	—					
0	2.5	0.03	10 ²	1	10 ²	10 ²	10 ²	0					—	—					
0	2.5	0.03	10 ²	1	10 ²	10 ²	10 ²	0					—	—					

TABLE 1—Continued

A. RESULTS OF TESTS ON THE BLOOD OF NON-PNEUMONIC SUBJECTS											B. RESULTS OF TESTS WITH HOMOLOGOUS PNEUMOCOCCI TYPES IN BLOOD TAKEN BEFORE TREATMENT FROM PATIENTS WITH PNEUMONIA										
Added to 0.5 cc. blood†			Pneumococcal action at 3 hours		Growth inhibition		Pneumococcal action at 48 hours		Phagocytic index		Added to 0.5 cc. blood†			Pneumococcal action at 3 hours		Growth inhibition		Pneumococcal action at 48 hours		Phagocytic index	
Sulfanilamide	Sulfapyridine	Serum	Inoculum	Growth	24 hours	48 hours	Inoculum	Growth			Sulfanilamide	Sulfapyridine	Serum	Inoculum	Growth	24 hours	48 hours	Inoculum	Growth		
mgm. per 100 cc.	mgm. per 100 cc.	units									mgm. per 100 cc.	mgm. per 100 cc.	units								
Pneumococcus Type V—1 Subject											Pneumococcus Type V—3 Patients										
0	0	0	10 ³	800	0	0	0	0			0	0	0	10 ³	25-150	0	0	0	0		1.5
10	0	0	10 ³	500	0	0	10 ⁴	0			10	0	0	10 ³	200	10 ⁴	0	0	0		
20	0	0	10 ³	1000	10 ⁴	10 ⁴	10 ⁴	0			100	0	0	10	25	10 ⁴	10 ⁴	10 ⁴	0		0.6
0	2.5	0	10 ³	800	10 ⁴	10 ⁴	10 ⁴	0			0	2.5	0	10	25	10 ⁴	10	10	0		0.6
0	5.0	0	10 ³	1200	10 ⁴	10 ⁴	10 ⁴	0			0	5.0	0	10 ³	600	10 ⁴	10 ⁴	10 ⁴	5		
0	10	0	10 ³	600	10 ⁴	10 ⁴	10 ⁴	0			0	5.0	0	10	25	10 ⁴	10 ⁴	10 ⁴	97		0.5
0	15	0	10 ³	600	10 ⁴	10 ⁴	10 ⁴	0			0	10	0	—	—	10 ⁴	10 ⁴	10 ⁴	0		
0	0	0.04	10 ⁴	17	10 ⁴	10 ⁴	10 ⁴	0	0.5		0	10	0	10	25	10 ⁴	10 ⁴	10 ⁴	17		0.9
0	0.4	10 ⁴	51	10 ⁴	10 ⁴	10 ⁴	10 ⁴	0	13.9		0	15	0	10 ³	140	10 ⁴	10 ⁴	10 ⁴	4		
0	4.0	10 ⁴	200	10 ⁴	10 ⁴	10 ⁴	10 ⁴	0	0.6		0	0	0.4	10 ³	25	10 ⁴	10 ⁴	10 ⁴	0		5.7
10	0	0.04	10 ⁴	35	10 ⁴	10 ⁴	10 ⁴	0	0.6		0	0	4.0	10 ⁴	60	10 ⁴	10 ⁴	10 ⁴	0		13.6
0	2.5	0.04	10 ⁴	61	10 ⁴	10 ⁴	10 ⁴	0	0.8		0	2.5	4.0	10 ⁴	30	10 ⁴	10 ⁴	10 ⁴	23		6.5
											0	2.74	0.4	10 ³	25	10 ⁴	10 ⁴	10 ⁴	5		7.6

Explanation: † All added materials were contained in 0.1 cc. saline. The figures represent the final concentration. H = Immune horse serum when not designated rabbit serum was used. — = Test not done.

Pneumococcal action. In the 3-hour test, except in some of the pneumonia patients, only 10³ and 10⁴ pneumococci were inoculated and agar pour plates were made after 3 hours' incubation. The numbers listed under growth indicate pneumococcus colonies in the pour plates. ∞ = too numerous to count. Smaller inocula than the ones listed showed no growth. With larger inocula the number of colonies were too numerous to count. Numbers in parentheses indicate the number of tests in which the same results were obtained. The result of each test is listed.

Growth inhibition. The figures represent the largest inocula showing no color change after incubation, or the extremes of the results of multiple tests.

Phagocytic index. Average number of pneumococci per polymorphonuclear leukocyte. + = Clumping of organisms and leukocytes preventing accurate count. When not listed the phagocytic index was under 0.5.

difference between the growth curves using this medium and those made with defibrinated blood did not indicate any striking inhibitory effect of the peptone on the action of sulfapyridine. This can be seen from a comparison of Figures 2 and 3 with Figure 9. Further growth curves with the same broth containing 2 per cent peptone and sulfapyridine 10 mgm. per 100 cc. were almost identical with those obtained with the 1 per cent peptone broth used routinely.

Action of sulfapyridine sulfanilamide and immune serum on pneumococci in fresh defibrinated human blood

Pneumococcal tests were done with Types I, III, and V pneumococci in the blood of non-pneumonic subjects and also in the blood taken before treatment from patients with pneumonia due to the homologous type of pneumococcus. Varying amounts of the drug and of homologous

immune serum were used. None of the bloods employed in these tests showed any pneumococcal action for the organisms used without the addition of these chemicals or serum.

The results shown in Table I clearly indicate the difference between the pneumococcal action resulting from the immune mechanism and that due to the effects of the drugs. In the 3-hour tests there is no reduction in the number of viable organisms except in the presence of immune serum. Furthermore, the end point in the pneumococcal tests as conducted was quite sharp when immune serum was present killing being complete at 48 hours with one of the decimal dilutions of culture while full growth occurred with the next higher dilution during this period. With the drugs on the other hand varying degrees of bacteriostasis and killing occurred with more than one of the dilutions of culture in which full growth did not occur.

As previously shown for the Type III pneumococcus (1), sulfanilamide exerted some bacteriostatic action with concentrations below 10 mgm per 100 cc of blood. With higher concentrations, this bacteriostasis is more marked and some bactericidal activity may occur. Each of the 3 types tested behaved similarly in this respect.

Sulfapyridine was considerably more effective than sulfanilamide in each instance. Bacteriostasis was noted with large inocula, and small numbers of organisms were killed in 48 hours in the presence of sulfapyridine in a concentration of 5 mgm per 100 cc. Concentrations of 10 mgm per cent resulted in complete killing of 10^4 pneumococci and there was a marked reduction in the number of viable organisms with larger inocula.

As previously shown by Ward (13, 14), the addition of immune serum resulted in considerable pneumococidal action, the degree varying with the amount of antibody added. It is of interest to note that definite pneumococidal action occurred with considerably lower concentrations than those required to induce phagocytosis by the method employed here.

In a number of tests, the smallest effective quantity of serum was combined with small amounts of sulfanilamide or sulfapyridine. The effect on the pneumococidal action of the blood was equivalent to a summation of the effects of each of these agents used separately.

The tests carried out in the blood obtained before treatment from patients with pneumococcal pneumonia gave results essentially similar to those obtained with the blood of the non-pneumonic subjects.

In Table I, the number of viable organisms present after 48 hours' incubation are recorded in detail. Where several tests were done with the same combination of materials, the results indicate the variations observed in different bloods. These data reflect clearly the inhibitory and bactericidal action of sulfapyridine.

The tests carried out with blood taken from patients after treatment gave similar results, corresponding to the concentration of antibodies and sulfapyridine present at the time. Where 3-hour tests were carried out in blood taken after treatment with sulfapyridine and no reduction in number of pneumococci was found at that time, the

final results were the same as those obtained with drug added to the original blood taken before treatment. These results are reported separately (2).

Effect of soluble specific substance Since homologous type-specific soluble substance is known to inhibit the pneumococidal action both of normal and of immune serum (14), it was of interest to see whether it would also interfere with the pneumococidal action of sulfapyridine. This was tested in the blood of a heavily infected patient with Type III pneumococcal pneumonia taken before treatment was begun and again after sulfapyridine therapy. The results of these tests are shown in Table II. The 3-hour tests indicated rapid killing of large numbers of added pneumococci in the presence of immune serum. This pneumococidal action of immune serum was inhibited in the presence of soluble-specific substance added in the form of a filtrate from a culture of the homologous pneumococcus. This neutralizing effect of the culture filtrate on the action of immune serum was evident even in the presence of sulfapyridine, since no killing occurred in 3 hours. The growth inhibition and pneumococidal action of the sulfapyridine, however, was not affected by the soluble-specific substance, as shown by the inhibition and killing at 48 hours.

DISCUSSION

The various growth curves indicate that, under the conditions of these experiments, growth of pneumococci occurred in the presence of concentrations of sulfapyridine up to 10 mgm per 100 cc in the same manner as in control media containing no sulfapyridine for an initial period of 4 hours or longer. During this early period there was usually no appreciable lag phase and the same logarithmic rate of growth occurred with sulfapyridine as without it. The growth phase was usually followed by a phase of stationary growth and then a period of maximum sulfapyridine effect. The rate of growth during the initial period, whether or not a stationary phase followed, and the duration of the latter when it occurred, seemed to depend on the strain used. The early growth phase was also longer at 27° C than at 37° C. Whether bacteriostasis or killing followed, appeared to depend on the population attained (which, in turn, depended on the size of

TABLE II

Effect of homologous soluble specific substance on the pneumococcal action of immune serum and sulfapyridine in defibrinated human blood

0.5 cc. patient's blood + 0.1 cc. broth containing			Pneumococcal action at 3 hours		Growth inhibition		Pneumococcal action at 48 hours	
Immune rabbit serum	Sulfapyridine to make final concentration	Pneumococcus (III) culture filtrate (SSS)	Inoculum	Organisms viable after 3 hours incubation	Largest inoculum showing no color change after		Inoculum	Organisms viable after 48 hours' incubation
					24 hours	48 hours		
cc.	mgm. per 100 cc.	cc.						
0	0	0	71	1,500±	0	0	7	∞
0	0	0.05	71	1,500±	0	0	7	∞
0.1	0	0	71 71,000	0	71,000	71,000	71,000	0
0.1	0	0.05	71	3,000±	0	0	7	∞
0	7.5	0	71	1,500±	71,000	71,000	71,000	0
0	7.5	0.05	71	2,000±	71,000	71,000	710 71,000	0 3
AFTER ADMINISTRATION OF 10 GRAMS SULFAPYRIDINE IN 44 HOURS								
0	8.0*	0	51	2,000±	51,000	5,100	51 510 5,100	0 10 600±
0	8.0*	0.05	51	2,000±	51,000	5,100	51 510 5,100	0 6 52
0.1	8.0*	0	51 51,000	0 200±	51,000	51,000	51,000	0
0.1	8.0*	0.05	51	1,200±	51,000	5,100	5 510 5,100	0 1 67

† The blood used in this experiment was from a patient with pneumococcus Type III pneumonia. First blood taken on the fifth day. Blood culture showed no growth.

* Concentration of free sulfapyridine in the blood (total = 9.9 mgm. per 100 cc.)

the inoculum), the concentration of the drug and the susceptibility of the strain. The rate of killing, when it occurred also depended on the same factors. Temperatures higher than 37° C may also enhance the action of sulfapyridine after the stationary phase. In defibrinated blood which originally lacked pneumococcal action the results were similar to those obtained with the artificial media used.

The principal feature exhibited in all the growth curves was that multiplication of the bacteria in the presence of sulfapyridine was essential before any bacteriostatic or bactericidal action of the drug became evident. Mere exposure to

the drug, as indicated by the results obtained at 5° C (Figures 10 and 11) was not enough to bring about the sulfapyridine effect. The lag in the action of the drug was apparently the same with concentrations of 5 and 10 mgm per 100 cc.

These facts are important in any study of sulfapyridine action. The use of large inocula (7) or the use of methods such as the slide-cell technique of Fleming (4) which permit colony formation during the early growth phase may result in misinterpretations concerning the degree of bacteriostasis or of bactericidal action attainable with the drug. Like Fleming we have noted small colony formation with faint hemolysis in agar plates poured with blood containing pneumococci and sulfapyridine during the active growth phase. We have also noted such small colonies in the blood of patients with massive bacteremia taken within a short interval after the injection of sufficient sodium sulfapyridine to raise the blood level to 10 mgm per cent. These colonies presumably represent multiplication during the early logarithmic phase of growth. The bactericidal effect of the sulfapyridine also is evident because we have not been able to recover viable organisms from such colonies on subcultures whereas normal looking colonies from the same plates grew freely when transplanted to fresh media.

Attainment of full growth is probably not the limiting factor in the action of sulfapyridine. This is borne out by a comparison of the curves obtained with large inocula of the stock Type III strain (Figure 3) and those obtained with the 'Taylor' strain (Figure 6). In the former the maximum populations reached were well below full growth and these were attained at 4 hours and the decline that followed occurred at about the same rate as in the curves without the drug (Figure 1). With the latter strain the curves which indicated that maximum growth was attained with the drug also showed more rapid declines in the population as compared with the controls growing without sulfapyridine.

Another interesting phenomenon which occurred with several of the strains is illustrated in Figures 6 and 8. With certain of the inocula the pneumococci were completely killed at 48° C in some experiments while at other times the population was greatly reduced during a period of 4 to 24 hours only to remain static again or

even to increase during the succeeding 24 hours or more. Whether or not this phenomenon was related to the development of drug-fast variants (10) within the population was not determined.

The results of the pneumococcal tests confirmed and extended the previous findings with respect to the effect of sulfanilamide on pneumococcus growth in human blood (1). This drug was shown to be bacteriostatic for the 3 types of pneumococci tested in concentrations up to 20 mgm per cent, and only occasionally was true bactericidal action noted within 24 hours when the higher concentrations were used. With sulfapyridine, bacteriostasis is effected by much lower concentrations. Even with this drug, however, more than 5 mgm per cent is necessary in order to insure the regular occurrence of bactericidal action even for small numbers of pneumococci.

Homologous immune serum was shown to induce pneumococcal action in fresh human blood. When used in small amounts in combination with small concentrations of sulfapyridine, the action is greater than when either agent is used separately. The action of the immune serum in such instances occurs early, as seen from the results of the 3-hour pour plates. The effect of sulfapyridine does not become evident for several hours.

Killing of the homologous type of pneumococcus occurs in the presence of sulfapyridine even in the blood taken before treatment from patients with massive bacteremia. Since certain amounts of the homologous soluble-specific substance are circulating in the blood of such patients, it would appear that the sulfapyridine action is not influenced by the presence of this substance. Even addition of further amounts of culture filtrate containing this substance in amounts sufficient to inhibit the action of added immune serum did not influence the action of sulfapyridine. These findings serve to explain, in part, why sulfapyridine may be effective even late in the course of pneumococcal infection and even in the presence of massive bacterial invasion of the blood. Except for the influence of focal purulent complications and perhaps other factors influencing the general reactions of the body, sulfapyridine should exert its beneficial effects in every case infected with a susceptible strain, provided the patient lives for several hours during which an effective concentration of the drug is maintained. Rapid destruc-

tion of circulating bacteria could be effected by the use of immune serum, but for this purpose complement and an adequate number of intact leukocytes are probably essential (15, 16).

SUMMARY AND CONCLUSIONS

Growth curves in a favorable artificial medium and tests with human blood originally lacking in pneumococcal properties indicated that sulfapyridine has considerable bacteriostatic and bactericidal action on pneumococci. The degree of bacteriostasis or of bactericidal action depends on the concentration of the drug and the number of pneumococci inoculated. Under the conditions of these experiments, growth invariably occurred before the sulfapyridine exhibited its effect.

Type-specific immune serum confers marked pneumococcal properties on fresh human blood originally lacking in such properties. Both the sulfapyridine and the immune serum are effective in normal human blood and in the blood of patients with pneumococcal pneumonia. The combination of small amounts of serum and sulfapyridine is more effective than either agent used separately in the same amounts. The destruction of pneumococci in the presence of immune serum is rapid whereas the action of sulfapyridine is considerably delayed.

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IMMUNOLOGICAL STUDIES ON PATIENTS WITH PNEUMOCOCCIC PNEUMONIA TREATED WITH SULFAPYRIDINE

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In a separate communication (1) we presented observations on the effect of sulfapyridine on the growth of Types I, III, and V pneumococci in a favorable artificial medium and in human blood to which it is added *in vitro* or which is obtained after its administration. The chemical was shown to have marked bacteriostatic and considerable bactericidal action on these three types of pneumococci. The mechanism of action of sulfapyridine was found to differ fundamentally from that of homologous types of specific immune serum. The action of the latter occurs only in the presence of phagocytic cells and is most effective in freshly shed blood. The action of the former is independent of the immune mechanism. Immune serum was found to exert its bactericidal effect rapidly whereas the action of sulfapyridine was considerably delayed and could not be demonstrated in any instance until after growth of pneumococci had occurred. When small amounts of immune serum were combined with low concentration of sulfapyridine, the bactericidal action of fresh blood occurred rapidly and was greater than when either of these agents was added separately in comparable amounts. In the present paper are presented the results of immunological studies in patients with pneumococcic pneumonia who were treated with sulfapyridine. These studies were carried out in an attempt to elucidate the relationship, if any, of the immune state to the mechanism of recovery from pneumonia in patients treated with this drug. Because of the wide variations in the antibody response of different patients to infections even of the same type and without serotherapy or chemotherapy and because of the paucity of available data, the results are presented for each type in considerable detail.

MATERIALS AND METHODS

The patients studied were all admitted to the medical wards of the Boston City Hospital during the 1938 to 1939 season. Only patients with clinical and x ray evi-

dence of pneumonia are included. In each instance a type specific pneumococcus was obtained from the sputum on one or more occasions. Blood cultures were taken before and at frequent intervals after therapy was begun. When positive cultures were obtained from blood or from infected exudates the type of pneumococcus found confirmed the sputum findings in each instance. The clinical aspects of the treatment are left for separate consideration and only relevant data are included. The immunological and chemical methods were the same as those used in other recent studies in this laboratory (1). Control tests with heterologous types were carried out in many instances. Most of these were negative and are not reported unless relevant. Blood was obtained for culture and for the various immunological tests before treatment with sulfapyridine or with serum was started. In almost every instance where these agents were used in sequence blood samples were taken before the administration of each was begun. Further samples of blood were taken at intervals after the onset of therapy. In those bloods taken during the course of sulfapyridine administration determinations of the concentration of this drug in the blood were done in addition to the immunological tests. Only a small number of cases treated with the combination of sulfapyridine and serum are included. The therapeutic serums used were mostly rabbit serums furnished by the Lederle Laboratories for clinical trial.

RESULTS

The bactericidal tests were carried out only in a limited number of cases of Types I, III, V, and VIII pneumococcic pneumonia and protection tests were done in cases with these types and also in a few Type II and Type VII cases. A total of 216 cases due to these 6 types was studied with protection and agglutination tests including 87 in which bactericidal tests were also done. The cases to be presented in the tables are selected as examples of the varieties of response noted with each of the types. The studies in the remaining types were limited to tests for agglutinins. The results will be presented separately for each of the 6 common types.

Type I Cases The results of the various tests in these cases are listed in Tables I and II and are shown graphically in Figures 1 and 2. Cases

TABLE 1

Results of immunological studies in patients with pneumococcus Type I pneumonia treated with sulfapyridine or with the combination of sulfapyridine and specific serum

Number	Sex and age	Therapy	First dose	Interval first to last dose	Amount	Results of tests									Termination	
						Day of disease	Blood culture	Blood sulfa pyridine		Growth inhibition	Pneumococci killed	Opsonic index	Mouse protection	Agglutinins	Mode	Day
								Free	Total							
			hours after onset	hours	grams			mgm per 100 cc		48 hours	at 48 hours					
1	M 12	S P	52	66	12	52 68 120	0 0 0	5 4	6 7	0 10 ⁸ 10 ⁸	0 10 ⁴ 10 ⁸	0 0 2 24 2	0 0 10 ⁴	0 0 0	Crisis	83
2	M 28	S P	84	96	21	75 84 102 9	+ 0 0 0	5 6	8 2	0 10 ⁸ 10 ⁷	0 10 ³ 10 ⁸	0 1 0 04 15 8	0 0 10 ⁸	0 0 2	Lysis	115- 131
3	M 37	S P	128	60	16	127 166 13	3 0 0	9 5	10 6	10 ³ 10 ⁷ 10 ⁴	10 ³ 10 ⁸ 10 ⁴	0 2 7 18 3	0 10 ⁸ 10 ⁸ × 10	0 4 32	Lysis	7-15
4	M 28	S P	32	96	21	18 32 65 100 7 8 14	0 0 0 0 0 0 0	6 4 9 8 3 5 Tr	6 4 11 6 5 1 Tr	10 ⁴ 10 ⁸ 10 ⁸ 10 ⁴	10 ⁴ 10 ⁸ 10 ⁸ 10 ⁸	0 24 0 0 10 4	10 ⁸ 10 10 10 ⁸ × 10 10 ⁸ × 50	0 0 0 32 64+	Crisis	84
5	F 69	S P Serum	98 101	30 23	13 580 th u	98 122 19	550 0 0	4 3	8 8	0 10 ⁷ 10 ⁸	0 10 ⁸ 10 ⁸	0 96 36 5 14 2		0 64+ 8	Lysis	131+
6	M 69	S P Serum	58 85	36 14	14 300 th u	58 84 118 13	109 + 0 0	2 7 3 2	4 9 7 1	0 10 ⁷ 10 ⁸	0 10 ⁷ 10 ⁸	0 36 6 3 18 5		0 0 32 16	Died	20
7	M 40	S P Serum	65 84	30 5	5 120 th u	65 84 106 9	0 0 0 0	4 0	5 2	10 ³ 10 ⁸ 10 ⁴	10 ³ 10 ⁴ 10 ⁴	0 0 14 27 5		0 0 8 4	Crisis	96
8	M 39	S P Serum	123 166	48 7	15 170 th u	112 121 166	21 1 0	3 2	5 8	10 ³ 10 ³ 10 ⁸	10 ³ 10 ³ 10 ⁸	0 0 1 1 1	10 10 10 ⁸	0 0 0	Died	174

Explanation of tables S P = sulfapyridine Tr = trace Serum Th u = thousand units

Day of disease and day of termination Wherever relevant, the number of hours after onset is indicated Italics indicate actual day of the disease

Blood culture + = growth of homologous type pneumococcus, 0 = no growth, numbers indicate colonies per cc of blood in agar pour plates

Growth inhibition Numbers represent largest inoculum which failed to show color change

Pneumococci killed Inoculum which yielded no growth in agar pour plates after 48 hours' incubation in blood

Protection Largest number of lethal doses protected by 0.2 cc of serum, 10⁸ × 4, 10⁸ × 10, etc. indicates protection against 10⁸ lethal doses by 0.2 cc of 1, 4, 10 dilution of the original serum, respectively

Agglutinins The greatest final dilution of serum showing floccular agglutination

Crisis Time when temperature first dropped below 100° F with improvement in symptoms

Lysis Same, with fever persisting

in which the data are inadequate are excluded from the tables but the individual observations are included in the figures. The results of tests made with blood obtained after serum therapy are excluded from the figures.

In Table I are listed the data on 8 patients who were studied with pneumococcal and phagocytic tests. These cases were chosen to illustrate both the variations occurring in the immune state before treatment as well as to give some indication of the variety of responses noted to treatment with sulfapyridine alone or to the administration of

TABLE II

Results of homologous protection and agglutination tests in additional cases of pneumococcus Type I pneumonia treated with sulfapyridine

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		Total dose	First dose	Interval first to last dose	Day of disease	Blood culture	Blood sulfapyridine		Mouse protection	Agglutination	Mode	Day
							Free	Total				
		grams	hours after onset	hours			mgm. per 100 cc.					
9	M 32	20	84	94	64 90 9 11	+	5.1 Tr	6.5 2.5	0 0 0 0	0 0 0 0	Critica	84
10	M 43	37	84	103	83 93 101 7 11 17	+	0 5.2 5.3	0 2.5 2.0	0 0 0 0	0 0 0 0	Critica	78
11	F 42	14	23	50	20 44 64 7	+	0 0 0	0 0 0	0 0 0	0 0 0	Critica	63
12	F 41	15	116	48	80 116 11 14	0	0 0 0	0 0 0	0 0 0	0 0 0	Lysis	127+
13	M 32	24	90	123	90 110 9	+	7.6 8.7	10 ⁴ 10 ⁴ ×4	0 0 0	0 0 0	Critica	106
14	M 43	23	76	126	76 112 8 10 13	+	5.8 6.6	0 0 0 0	0 0 0 0	0 0 0 0	Critica	110
15	M 35	16	123	84	118 123 134 8 10 13 17	0	0 0 0	0 0 0	0 0 0	0 0 0	Critica	144
16	M 33	37	74	126	81 74 7 9 11 14 17 23	+	0 0 0	0 0 0	0 0 0	0 0 0	Lysis	8

TABLE II—Continued

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		Total dose	First dose	Interval first to last dose	Day of disease	Blood culture	Blood sulfapyridine		Mouse protection	Agglutination	Mode	Day
							Free	Total				
		grams	hours after onset	hours			mgm. per 100 cc.					
17	M 43	18	20	84	20 29 56 114 8 10	0	2.4 4.5 0.5	4.3 4.8 0.9	0 0 0	0 0 0	Lysis	54+
18	F 40	53	110	264	110 8 7 9 11 14 16 18 24	32	2.7 8.0 10.4 8.0 6.4 5.8 Tr	3.2 3.3 11.8 9.8 5.4 0.8 Tr	0 0 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10	0 0 0 0 0 0 0	Lysis	8-24
19	M 36	10	53	84	53 64 80 128 8 13	2	2.1 2.2 2.8 0	2.1 4.8 5.4 0	0 0 10 ⁴ 10 ⁴ 10 ⁴ 10	0 0 0 0 0 0	Critica	84
20	M 47	31	74	84	74 104 123 7 9 18	0	3.8 3.1 3.5 Tr	6.3 6.3 6.3 Tr	0 0 10 10 ⁴ 10 ⁴ 10 ⁴	0 0 0 0 0 0	Critica	83
21	M 41	23	123	108	123 145 166 9 11 14	0	2.8 1.6 2.3 4.8	4.4 4.4 4.7 10.5	0 0 10 ⁴ 10 ⁴ ×4 10 ⁴ ×4 10 ⁴ ×4	0 0 0 0 0 0	Lysis	8
22	M 28	20	48	48	48 72 100 124 18	0	2.7 4.0 Tr	12.7 7.8 1.5	0 0 10 ⁴ 10 ⁴ 10 ⁴	0 0 0 0 0	Lysis	54-140
23	M 77	24	52	106	53 67 115 9	0	2.3 10.3	3.9 11.4	0 10 ⁴ 10 ⁴ 10 ⁴	0 0 0 0	Critica	74
24	F 53	23	112	120	112 124 8 8 10	600 +	2.8 10.8 14.9 12.4	3.5 12.4 18.7 15.4	0 0 10 ⁴ 10 ⁴ 10 ⁴	0 0 0 0 0	Died	10

the combination of sulfapyridine and serum. Some of the patients had pneumococcal activity in their blood before treatment. In some instances (Numbers 4 and 8) mouse protective antibody was demonstrated before the institution of therapy. In the control tests done on the bloods taken before treatment when inhibition of growth or killing took place both occurred to the same degree and this was irrespective of the presence of protective antibodies or agglutinins. This parallelism between growth inhibition and killing was also evident when other antibodies (protec-

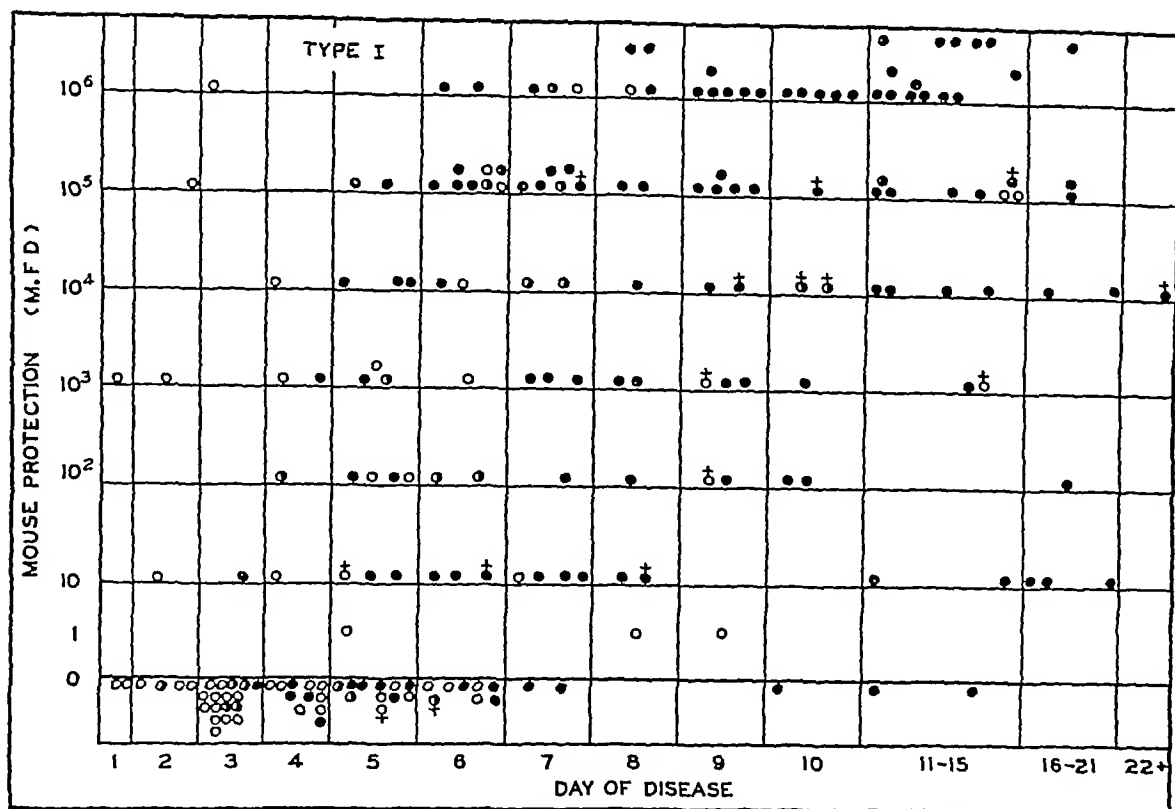


FIG 1

See Figure 4 for explanation

tion, agglutinins, opsonins) were acquired either spontaneously or after the administration of specific serums. In the bloods taken following sulfapyridine therapy, the extent of inhibition and of killing was not always parallel in the absence of other antibodies. This lack of parallelism is even more evident with respect to growth inhibition after 24 hours, as will be seen later. While growth inhibition and killing occurred following sulfapyridine therapy in the absence of other antibodies, these activities occurred to a considerably greater extent in the presence of the other antibodies.

Four patients who were treated with the combination of sulfapyridine and serum are included in Table I. One of these (Number 5) had a severe infection with a massive bacteremia, serum and sulfapyridine were started almost simultaneously, a balance of antibodies was rapidly established and maintained and the patient recovered. In the other 3 patients specific serum was given at varying intervals after therapy with sulfapy-

ridine was started because the clinical response was considered inadequate. Two of these 3 patients died. At the time serum was started in one of these cases (Number 6) the concentration of sulfapyridine in the blood was low, bacteremia was still present and extension of the pulmonary lesion had occurred. Pneumococcal tests were not done immediately before the administration of serum in this case but a high titer of antibodies was established and maintained after serum was given, and the drug had to be omitted because of excessive vomiting. The patient developed empyema and died at the end of the third week of illness with a complicating hemolytic streptococcus septicemia. In the other fatal case (Number 8) a rapid downhill course continued after sulfapyridine was given. Serum treatment was undertaken when the patient was in extremis and death occurred shortly thereafter. In spite of the unfavorable course in this case it was found that protective antibodies and some opsonins had developed at the time serum treatment was insti-

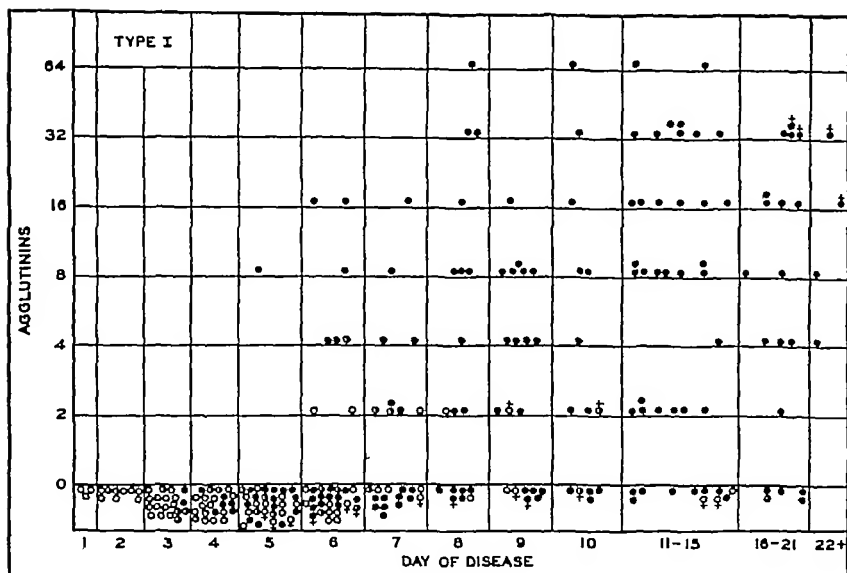


FIG. 2

See Figure 4 for explanation

tuted. The blood of the fourth patient (Number 7) had pneumococcal power before treatment and this increased after sulfapyridine and before serum was given. Crisis occurred 12 hours after the first dose of serum and 31 hours after the first dose of sulfapyridine. The relation of pneumococcal activity to the presence of agglutinins and protective antibody was the same regardless of whether the latter antibodies were injected or actively acquired.

The results of tests for mouse protection and agglutinins in 16 other patients are given in Table II. Here it is seen more clearly that these antibodies do not appear coincident with sulfapyridine-induced crises. This is particularly true with respect to the agglutinins. Patients in whom mouse protection was present before treatment are excluded from this table but the results of such tests are given in the figures.

A study of Figures 1 and 2 indicates that mouse protective antibodies were infrequent before the fifth day of the disease and agglutinins rarely oc-

curred before the sixth or seventh day. When such antibodies were present early they occurred with similar frequency in bloods obtained before or after treatment. For example on the sixth day (Figure 1) 7 of 12 patients tested either before or within 24 hours of the beginning of treatment had protective antibodies. On this same day 11 of 14 patients who had already received therapy for more than 24 hours had mouse protective antibodies. All of the tests done on the seventh day or later, but before treatment, showed the presence of some protective antibodies. In most of the patients in whom treatment was begun after the seventh day agglutinins were not found in the blood before treatment. These were severe cases and most of them died. Recovery was associated with the development of protective antibody in each instance except in Case 12. Agglutinins were not demonstrated in this and in 2 other cases (15 and 24). In the latter there was a massive bacteremia before treatment, empyema developed and death occurred after the blood

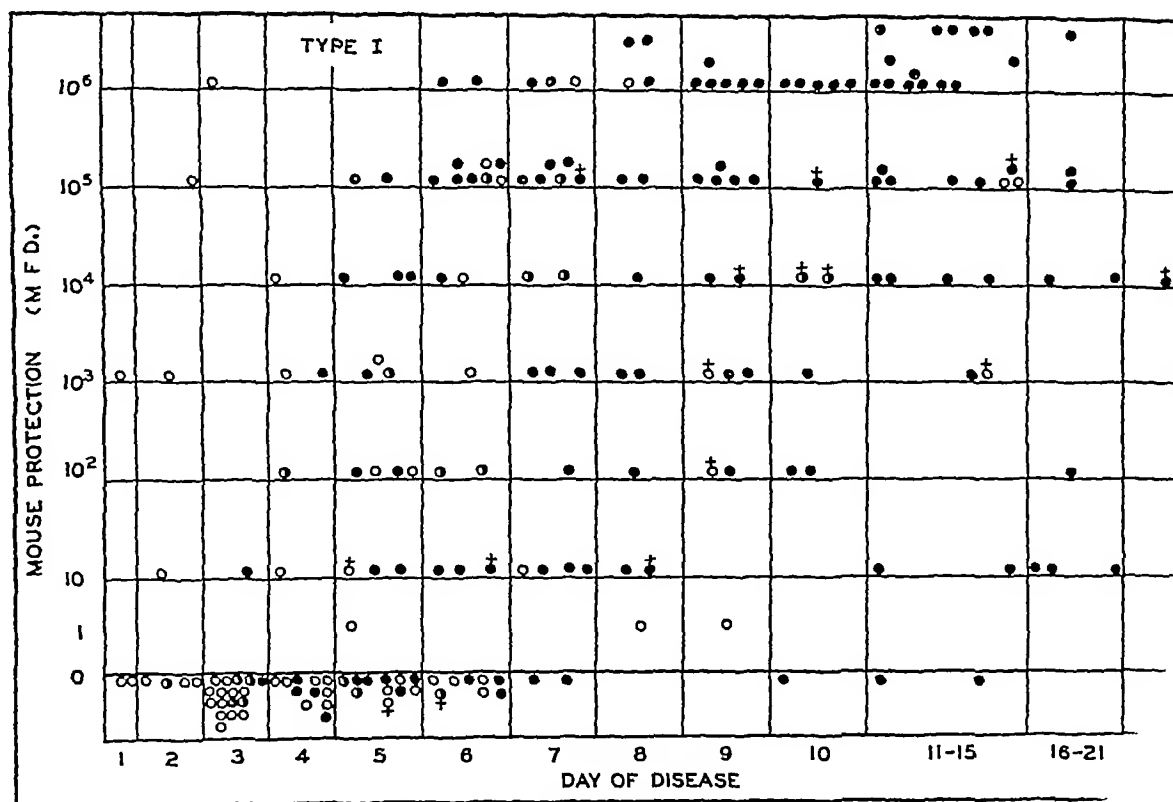


FIG. 1

See Figure 4 for explanation

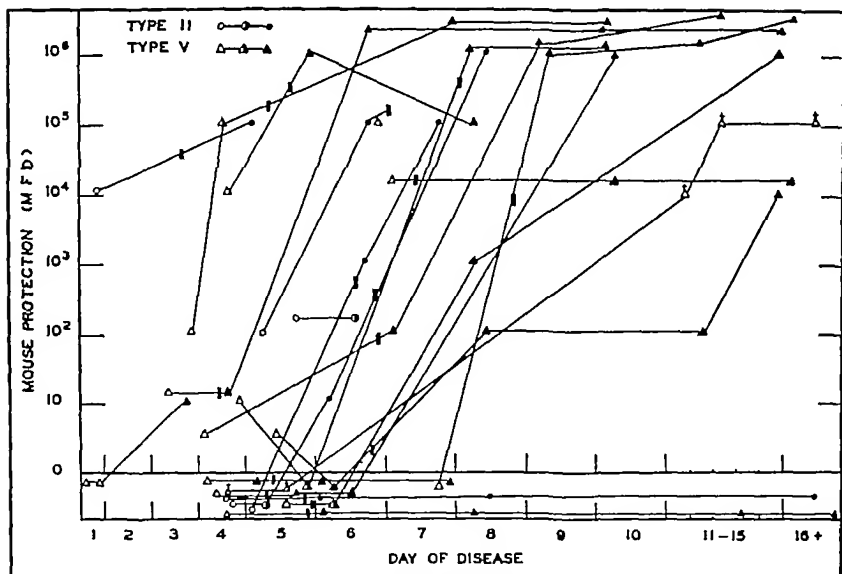


FIG. 3

See Figure 4 for explanation

case before treatment are noted in the table. These results may be interpreted as indicating that large amounts of antigen were neutralized in the blood temporarily by the addition of serum. This probability is further suggested by the drop in the agglutinin titer during the 8 hours between the 2 tests done after treatment. The preliminary serum showed a very dense precipitate when homologous antiserum was added—even with high dilutions of both the patient's serum and the specific Type II antiserum. Studies similar to the ones presented here have in the past been carried out on patients treated without sulfapyridine and have been previously reported (2 3 4)

Type V Cases In Table IV are listed the results in 6 patients in whose blood bactericidal and opsonic tests were carried out. These included 3 patients with various titers of mouse protective antibody in serum obtained before treatment. This protection was associated in each instance with moderate to high bactericidal titers. The 3 patients whose serum lacked mouse protection be-

fore treatment also had no appreciable pneumococcal activity in the blood at this time. Protection was present in some instances early in the disease without high titers of opsonins (Cases 4, 5). In Case 1 neither protection nor agglutinins developed during the period of observation. At the beginning of the seventh day however this patient had good pneumococcal activity and opsonins in his blood in the absence of circulating sulfapyridine. Such action was not demonstrable in the control blood taken before treatment.

Of 8 other Type V Cases which are shown in Table V the serum of 2 had protection against 1000 or more fatal doses and in 1 there was protection against 10 fatal doses before treatment. Protective antibody in all the cases developed or increased on the sixth day or later and in every instance this occurred after crisis (Figure 3). Agglutinins usually appeared or increased after the sixth day (Figure 4).

Serum treatment supplemented sulfapyridine in 3 of the 14 cases shown in the tables. In Case 6

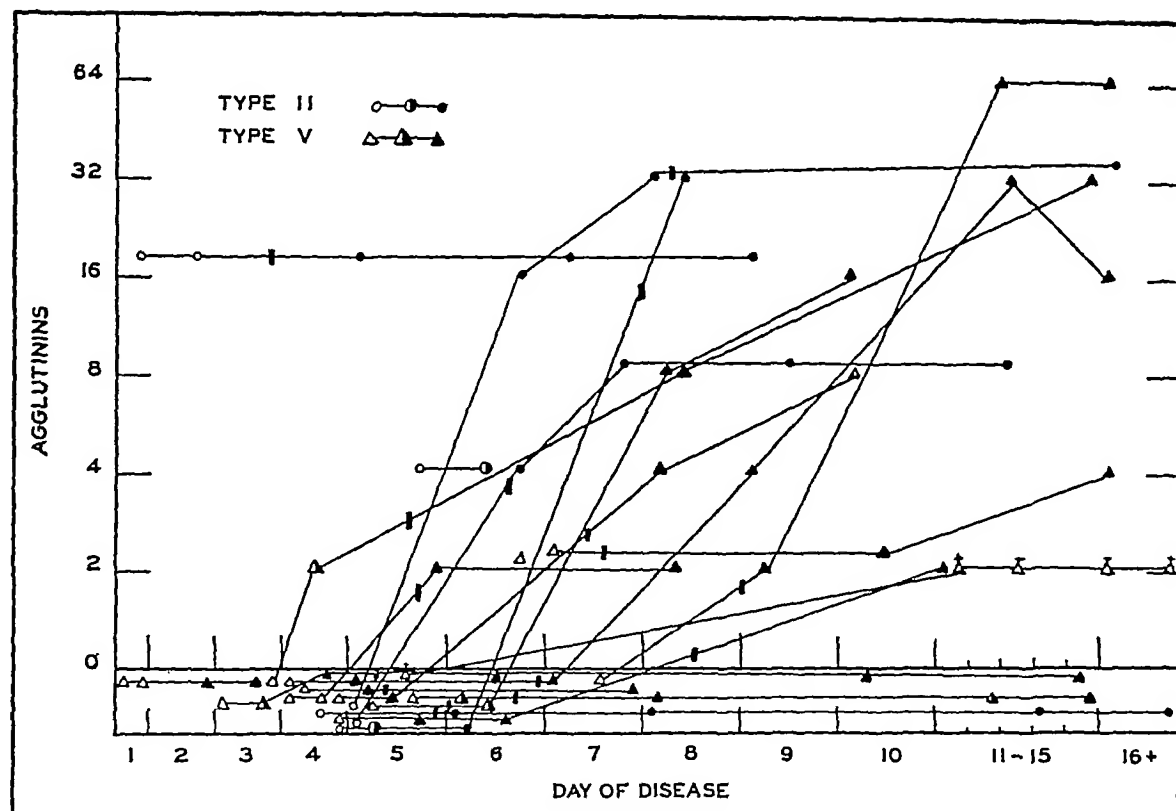


FIG 4

EXPLANATION OF THE FIGURES

In Figures 1 to 4 open circles or triangles represent tests done before any sulfapyridine treatment was given, solid ones represent observations made more than 24 hours after the first dose of sulfapyridine and the ones half open and half solid represent observations made during the first 24 hours of treatment.

The solid lines serve to connect the tests made in individual patients. These lines are omitted for the sake of clarity from the charts of the Type I cases. The solid bar indicates the time of crisis.

Observations made after serum treatment are omitted
 + = fatal case.

the patient showed less fever following sulfapyridine therapy. The drug was not well tolerated and the patient again appeared ill for about 36 hours after drug was discontinued. He was then given serum and became afebrile and symptom-free within 8 hours after the first injection. The other 2 cases appeared to be severely ill when treatment was begun and both serum and drug were started about the same time. Neither of these 2 patients had blood stream invasion before treatment, indeed, one had a good titer of antibodies at the time.

More detailed titrations of the amount of protective antibody present after crisis were made in the serum of 4 patients treated with sulfapyridine

alone and, for comparison, in 2 other patients shortly after specific serum treatment. Only bloods in which 0.2 of a cc of serum protected against 1,000,000 fatal doses in preliminary tests were chosen for these more detailed titrations. In the 2 patients treated with specific serum (Numbers 6, 14) the titrations showed 125 and 250 units per cc. of blood 16 and 18 hours, respectively, after the first injection of serum. Each of the 4 non-serum treated cases had 20 units or more. In Case 7, the titer rose from 20 to 125 units and in Case 10 from 50 to 250 units after the ninth and twelfth days respectively.

Previous studies on the antibody response of patients with Type V pneumonia treated without

TABLE IV

Immunological tests in patients with pneumococcus Type V and Type VIII pneumonia treated with sulfapyridine alone or with specific serum

Number	Sex and age	Therapy	First dose	Interval first to last dose	Amount	Results of tests										Termination	
						Day of disease	Blood culture	Blood sulfa pyridine		Growth inhibition	Pneumococci killed	Opsonic index	Mouse protection	Agglutinins	Mode	Day	
								Free	Total								
			hours after onset	hours	grams			mgm	per 100 cc	48 hours							

TYPE V CASES

1*	M 25	S.P.†	77	60	18	77 100 121 168	0 0 0	5.4 4.6 0	6.9 7.9 0	0 10 ⁴ 10 ⁴ 10 ⁴	0 10 ⁴ 10 ⁴ 10 ⁴	0.14 0 0.22 8.4	0 0 0 0	0 0 0 0	Crisis	106
2	M 44	S.P.	74	156	37	74 111 135 11	0 0 0	3.5	5.6	10 10 ⁴ 10 ⁴ 10 ⁴	10 10 ⁴ 10 ⁴ 10 ⁴	0.74 0 0 36.4	0 0 0 10 ⁴	0 0 0 2	Lysis	5-8
3	M 53	S.P.	96	78	20	96 107 8 10	0 0 0	4.5 Tr	4.7 Tr	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	1.6 1.3 0 10 ⁴	10 0 0 10 ⁴	0 0 0 8	Crisis	7
4	F 32	S.P.	86	84	19	86 96 120	0 0 0	5.3 5.6	5.7 7.2	10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴	0.96 0.60 0.8	10 ⁴ 10 ⁴ 10 ⁴	0 0 2	Crisis	115
5	M 52	S.P.† Serum	140 146	96 11	31 320 th.u.‡	140 146 160 13	0 0 0	0 1.3 5.4 0	0 3.3 9.9 0	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0.44 2.5 14.6 7.3	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	2 32 16	Lysis	8-17
6	M 66	S.P. Serum	20 72	18 4	8 160 th.u.	20 43 65 88 10	0 0 0	5.0 Tr 0	9.5 2.9 Tr	0 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	1.5 0.88 0.70 15.4 +	0 0 10 10 ⁴ ×25 10 ⁴ ×10	0 0 0 16 8	Lysis	38-80

TYPE VIII CASES

1	M 49	S.P.	62	84	16	62 81 7 11	0 0	6.7	8.3	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0.90 0 0 1.9	10 0 10 ⁴ 10 ⁴	0 0 0 0	Crisis	72
2	M 33	S.P.	28	108	18	28 40 92 145 9	+ + 0	4.7 5.9	5.4 6.2	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0.48 0 0.68 1.2	0 0 0 10	0 0 0 2	Crisis	31

* Pn. III and V in sputum. No antibodies for Pn. III developed.

† S.P. = sulfapyridine

‡ Sulfanilamide given in first 3 days of illness at home.

§ th.u. = thousand units.

TABLE V

Additional patients with pneumococcus Type V pneumonia treated with sulfapyridine

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sulfa pyridine		Mouse protection	Agglutins	Mode	Day
							Free	Total				
		hours after onset	hours	grams			mgm per 100 cc.					
7	M 21	75	50	9	75 99 140 9 13	0	Tr Tr Tr	1.3 4.9 Tr	1 0 10 ³ 10 ³ ×4 10 ³ ×25	0 0 0 4 32	Crisis	141
8	M 50	103	60	17	103 121 171 12 15	0 0 0 0 0	3.9 4.7	4.2 5.3	1 0 10 ³ 10 ³ 10 ⁴	0 0 0 0 0	Crisis	135
9	M 55*	103	72	18	103 127 178 15	0	2.0 4.6	3.8 8.3	0 0 10 ³ 10 ³ ×4	0 0 8 32	Crisis	120
10	M 63	166	132	41	166 202 18 16	0 0	5.9	10.0	0 10 ⁴ 10 ⁴ ×10 10 ⁴ ×50	0 2 64+ 64+	Crisis	101
11	M 49	52	84	25	52 91 138 10 15	0 0 0 0 0	8.7 8.3	10.0 9.5	10 10 10 ⁴ 10 ⁴ + 10 ⁴ +	0 0 0 0 0	Lydia	61-85
12	F 50	148	80	15	148 11 16	0			10 ⁴ 10 ⁴ 10 ⁴	2 2 4	Crisis	153
13	M 41	72	84	21	70 84 158 10	0 0 0 0	3.0 1.4	4.4 3.5	10 ³ 10 ⁴ 10 ³ ×4 10 ³ ×4	0 2 16 16	Crisis	102
14	M 65	11	8	44	1 +18 +5	0 0 0	4.8 Tr	8.7 2.4	0 10 ³ ×50	64+ 32	Crisis	+18

* Pn XIII also found in sputum All sera agglutinated Pn XIII in 1:4 dilution

† Serum treatment begun 3 hours later, 320,000 units given within 4 hours

serums or active chemicals have been reported from this laboratory (4)

Type VII Cases In this type as in the Type II Cases, pneumococcal tests were not carried out because of the high titers regularly demonstrable before and during the course of treatment. Protective antibodies, as previously pointed out (5, 6), are also frequently present in high titers early in the course of pneumonia due to this type of pneumococcus. The same is not true, however, for homologous agglutinins. Most of the patients in whom blood taken before sulfapyridine treatment showed protective antibody were

not followed further and are not shown in the table

In Table VI are listed the results of protection and agglutination tests in 15 cases of Type VII pneumococcus pneumonia. Eight of these patients had protective antibodies in various titers in the early serum taken before treatment. In the 5 patients whose blood showed no protection before therapy, such antibodies appeared in appreciable titers only after the sixth day. This was after the time of crisis in each instance. Agglutinins appeared on the sixth day or earlier in only 2 of the 13 patients who did not receive serum and 1 of these 2 patients had protective antibody prior to treatment.

Serum treatment supplemented chemotherapy

TABLE VI

Patients with pneumococcus Type VII pneumonia treated with sulfapyridine

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sulfa- pyridine		Mouse protection	Agglutins	Mode	Day
							Free	Total				
1	M 15	41	132	15	41 60 159 10	0 0	3.0 3.1	3.6 3.9	0 0 10 ⁴	0 0 4 16	Crisis	127
2	M 82	52	84	20	50 64 88 7 8	0	Tr 3.1 0	1.7 5.7 0	0 10 ⁴ 0	0 0 8 64	Crisis	60
3	M 24	61	78	16	61* 99 146 9	0 0	4.1 3.2	4.4 3.8	0 10 10 ⁴ 10 ³	0 0 2 4	Crisis	92
4	F 54	183	72	18	183 205 253 14	0 0	6.3 10.1	8.4 12.6	0 10 ³ 10 ⁷ 10 ⁷	0 0 4 64+	Crisis	200
5	F 40	174	54	10	174 18 15	0	Tr	2.2	0 10 ⁴ 10 ⁴	0 2 2	Crisis	102
6	F 34	9	84	22	8 10 18 14 17	0 0	7.5 4.5	10.5 8.1	10 ⁴ 10 ⁷	0 8 32 64	Crisis	11
7	M 57	136	8	26	136 148 198 11	0 0 0	4.2 9.2	4.2 11.2	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 0 8 8	Crisis	184
8	M 26	54	84	18	54 89 144 10 15	0 0	9.4 6.2	10.0 8.9	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 4 8 32 64	Crisis	61

TABLE VI—Continued

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sulfapyridine		Mucosa protection	Agglutinin	Mode	Day
							Free	Total				
		hours after onset	hours	grams			mgm. per 100 cc.					
9	M 67	111	66	14	111 122 4 18 18	0 0 0 0 0	3.4	4.8	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 0 18	Lysis	8-8
10	M 67	175	78	22	173 10 13 18 18	0	11.7	13.3	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 4 4 4 0	Crisis	183
11	M 34	101	11	5	101 139 18	0 0 0	2.1	2.9	10 ⁶ 10 ⁶ 10 ⁶	0 0 0	Lysis	4-17
12	M 50	83	84	20	84 8 10 10	0 0 0 0	9.3	11.4	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 16 18	Lysis	103-118
13	M 57	153	40	9	153 203	0 0	1.8	4.7	10 ⁶ 10 ⁶	0 0	Died	231
14	F 33	58	48	9	48 58 701 123	+	1.3	2.6	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 18	Lysis	75-140
15	M 35	153	96	21	153 233 2631	+	7.7 6.9	9.5 9.1	10 ⁶ 10 ⁶ 10 ⁶	0 0 0	Died	278

* Blood culture taken 7 hours before was positive

† Serum treatment begun 4 hours later 360 000 units given in 19 hours.

‡ Hematuria leukocytosis (55 000) and severe toxic symptoms after sulfapyridine. Serum treatment begun at this time 100 000 units given in 2 hours. Transfused 2 days previously

in 2 of the cases. In Case 14 bacteremia was present but it had already ended before treatment with the drug was begun. Serum therapy was started at a time when the patient's temperature had already dropped, the blood had remained sterile but the symptoms and other signs of activity and progressive pneumonia were still present. Recovery was slow even after serum administration. In Case 15 21 grams of sulfapyridine had been given in the course of 4 days without affecting the bacteremia or the pneumonia. Severe toxic symptoms from the drug, including hematuria and marked leukocytosis developed. Serum treatment was finally undertaken but the patient died within a few hours after the first dose.

Type VIII Cases The results of the antibody studies in 15 patients with pneumococcus Type

TABLE VII

Additional patients with pneumococcus Type VIII pneumonia treated with sulfapyridine

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sul- fapyridine		Mucosa protection	Agriculides	Mode	Day
		hours after onset	hours	grams			Free	Total				
3	F 27	62	84	16	54 97 9 18	0	3.8	5.3	0 10 ⁶ 10 ⁶	0 0 18 16	Crisis	91
4	M 49	63	84	16	62 51 151 11	0	0.7	8.3	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 0	Crisis	72
5	F 30	129	144	29	129 183 200 18 16 19	0	7.1 11.7 5.6	7.7 13.3 6.3	0 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 3 8 8 16	Crisis	157
6	M 33	28	106	15	28 42 44 144 9	+	4.7 3.5	5.4 5.9	0 0 0 0 0	0 0 0 0 2	Lysis	31-78
7	M 77	18	96	28	18 13 14 10 10 34	0	9.1 13.5 1.5	10.3 15.5 2.9	0 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 0 0 22	Lysis*	18+
8	M 34	41	1†	8	42 30 30 7 11	0	12.3	12.8 0	0 0 0 0 10 ⁶	0 0 0 0 0	Lysis	82-94
9	M 63	†	73	17	† 60 † † 11	0	4.4	4.6	0 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 4 4	Lysis	+4
10	F 44	55	64	17	52 115 8	0	8.3	7.7	0 10 ⁶ 10 ⁶	0 0 3	Crisis	64
11	M 19	39	73	21	23 30 93 128 9	0	6.7 4.1	8.7 5.4	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 4 8 8	Crisis	48
12	M 69	48	48	11	48 66 92 122	0	8.3 9.3	9.1 11.1	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 4	Crisis	64
13	M 40	150	96	25	150 9 11 18 18	0	4.2 2.4	6.2 4.0	10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶ 10 ⁶	0 0 0 2 0	Lysis	100-176
14	M 51	129	103	23	127 166 174	0	10 ⁶	+	10 ⁶ 10 ⁶ 10 ⁶	18 23	Crisis	137
15	F 53	110	48	18	110 119 19	0	8.2	5.3	10 ⁶ 10 ⁶ 10 ⁶	0 0 4	Crisis	129

* Abscess of leg and suppurative otitis media. Marked excitement during treatment. Hematuria.

† Single dose of 5 grams of sulfapyridine in 50 per cent dextrose solution intravenously (7)

VIII pneumonia are shown in Tables IV and VII. Bactericidal tests were done before treatment in a number of cases but all of the bloods tested showed marked pneumococidal properties at this time and further tests were not carried out in most instances. The results in 2 cases in which the tests were carried through after treatment are shown in Table IV. These results were essentially the same as in the cases of other types.

Mouse protective antibody was demonstrated

before treatment in 7 cases. In 5 of these patients, however, the blood protected against only 10 lethal doses early in the disease and this titer either remained the same or was no longer demonstrable in a later test. In the 2 patients who showed protection against 100 and 10,000 fatal doses (Numbers 13, 14) the earliest tests were done on the seventh and sixth days, respectively. Protection developed in all the other patients in various degrees and at the usual time. Agglu-

TABLE VIII

Patients with pneumococcus Type III pneumonia treated with sulfapyridine or with the combination of sulfapyridine and specific serum

Number	Age and sex	Therapy	First dose	Interval first to last dose	Amount	Results of tests										Termination	
						Day of disease	Blood culture	Blood sulfa-pyridine		Growth inhibition	Pneumo-cocci killed	Opsonic index	Mouse protection	Agglutinations	Mode	Day	
								Free	Total								
			hours after onset	hours	grams			mgm per 100 cc									
1	F 51	SP	117	144	31	116	+			0	0	0.16	0	0	Crisis	161	
						140	0	11.8	12.9	10 ⁶	10 ⁶	0.02	0	0			
						168	0	10.5	12.3	10 ⁶	10 ⁴	0	0	0			
						9	0	8.1	9.6				0	4			
						10	0	9.1	10.5	10 ⁶	10 ⁶	1.4	10 ³	32			
						14	0			10 ⁶	10 ⁶	++	10 ⁴	16			
2	M 42	SP	116	30	10	116	0			0	0	0.9	0	0	Lysis	7-14	
						137		6.2	8.3	10 ⁴	10	0.5	0	0			
						161		8.0	9.9	10 ⁴	10 ³	0.5	0	0			
						10				10 ⁴	10 ⁴	0.5	0	0			
						13							0	0			
						16				10 ⁴	10 ⁴	0	10	2			
3*	F 46	SP	109	148	20	108	0			0	0	0	0	0	Crisis	123	
						120	+	4.5	5.0	10 ³	0	0.02	0	0			
						8	0	9.0	11.2	10 ⁶	10 ⁶	0.04	0	0			
						13	0	4.3	5.5	10 ⁶	10 ⁵	0.3	10	4			
4	F 48	SP	58	84	17	58	0			0	0	0.02	0	0	Crisis	71	
						71	0	4.3	4.3	10 ⁶	10 ³	0	0	0			
						120		9.5	12.9	10 ⁶	10 ⁶	0	0	0			
						9		Tr	Tr	10 ³	10 ³	0.28	0	2			
						12				10 ³	10 ³	0.32	0	0			
5†	M 72	SP	89	104	25	89	0			0	0	0.22	0	0	Crisis	109	
						98	0	3.2	4.6	10 ⁵	0	0.32	0	0			
						123	0	5.0	9.0	10 ⁴	10 ³	0.20	0	0			
						11		0	0	0	0	0	0	0			
6†	F 69	SP	170	132	38	170	0						0	0	Died	39	
						180	+	2.5	2.5					0			
						10	0	7.8	8.7	10 ⁶	10	1.1					
						16	0	2.0	2.8	10 ⁵	10	0	10	0			
						20	0			0	0	0.08	0	0			
						31							0	0			
7	M 69	SP	46	7	4	46	0			10 ⁵	10 ³	0.08	0	0	Crisis	72	
						66		3.5	4.2	10 ⁶	10 ⁶	0.08	10 ³	2			
						9							10 ⁴	32			
						14							10 ⁶	32			

TABLE VIII—Continued

Number	Age and sex	Therapy	First dose	Interval first to last dose	Amount	Results of tests										Termination	
						Day of disease	Blood culture	Blood sulfa pyridine		Growth inhibition	Pneumococci killed	Opsonic index	Meningeal protection	Agglutination	Mode	Day	
								Free	Total								
			hours after onset	hours	grams			mgm. per 100 cc.									
8	F 37	S.P	36	72	18	35 57 7 10	0 0 0 0	1.5 0	3.8 0	10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴	0.46 0.16 5.1	10 ⁴ 0 10 ⁴ 10 ⁴	2 2 16 64	Crisis	48	
9†	M 55	S.P	146	60	15	145 165 189 212 11	1 0 0 0 0	6.8 6.9 6.9 0	8.7 10.1 11.1 3.7	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0.08 0.68 1.0 2.1 1.6	10 10 10 10 ⁴ 10 ⁴	0 8 10 64 64	Crisis	185	
9a		S.P	66	72	18	65 89 142	0 0 0	5.7 2.9	8.6 4.8	10 10 ⁴	10 10	0.16 0.26	0 0 0	0 0 0	Crisis	77	
10	M 43	S.P	4 19	72 9	18 45	4 30 12 21 29	0 0 0 0 0	2.1 5.3	5.2 6.9	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 0.04 0 0.04 0.26	0 0 0 0 0	0 0 0 0 0	Lysis	3-38	
11	M 75	S.P Serum	105 109	156 6	32 500 th.u.	105 124 17	0 0 0	6.6	6.8	0 10 ⁷ 10 ⁴	0 10 ⁴ 10 ⁴	0 0.7 0.3	0 10 ⁴ 10 ⁴	0 32 2	Crisis	155	
12†	M 56	S.P Serum	50 66	84 31	19 180 th.u.	50 66 84 111 13 16	0 0 0 0 0 0	7.1 4.5 6.7	9.0 5.5 7.4	0 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 10 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 0 2.1 2.9	0 0 10 ⁴ 10 ⁴ 10 ⁴ 10	0 0 2 8 2 0	Died	17	
13**	F 64	S.P Serum	153 154	17 4	47 160 th.u.	153 8 12 26 35	0 0 0 0 0	5.6 0	8.8 0	10 ⁴ 10 ⁷ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0 0.4 2.5 3.5	0 10 ⁴ 10 ⁴ 10 ⁴	0 8 16 4 4	Lysis	7-25	
14	F 50	S.P Serum	30 55	72 4	15 60 th.u.	30 51 75 9 16	0 0 0 0 0	7.9 5.2	10.0 9.3	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴ 10 ⁴	0.10 0.06 + +	0 0 10 ⁴ 10 ⁴	0 0 8 16 32	Crisis	68	

* Irrational 2nd to 4th day after crisis.

† No protection with homologous strain in each instance

‡ Developed persistent nitrogen retention and edema after 5 days of treatment

§ Same results in opsonic and killing tests with blood culture strain

|| Recurrence after 6 months. No protection with sputum strain.

** Alcoholism and delirium tremens.

*** Drug given intermittently

tumins were not demonstrable in 2 of the patients (Numbers 1 and 4) the last observation having been made on the eleventh day in each instance. These antibodies usually did not appear until the seventh day or later. In 1 of the 2 cases in which agglutinins were found on the fifth day or earlier

(Number 9) the time of onset was not well defined and may have been several days earlier than is indicated in the chart. In Case 8 the only specific therapy consisted of an intravenous injection of a glucose sulfapyridine solution containing the equivalent of 5 grams of sulfapyridine. Thus pa-

tient improved gradually and was symptom free within 28 hours after the injection but protective antibodies did not appear until 8 days after the patient was afebrile. The solution used was probably inert when given intravenously (7).

Previous studies of the antibody response in patients with pneumococcus Type VIII pneumonia treated without serum or sulfapyridine have been previously reported (8). They are in essential agreement with the present findings.

Type III Cases As shown in previous studies on patients treated without serums or drugs (8),

TABLE IX

Additional patients with pneumococcus Type III pneumonia treated with sulfapyridine

Number	Sex and age	Sulfapyridine therapy			Results of tests					Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sulfa pyridine	Mouse protection*	Agglutins	Mode	Day
		hours after onset	hours	grams			mgm. per 100 cc.				
15	M 58	110	32	9	110 142	0	3.6	0 10 ²	0 8	Lysis	5-8
16	M 50	50	9	41	50 74 121 164 10	0 0 0 0 0	9.0 10.1 Tr 8.5	10 ² 10 ² 10 ² 10 ²	0 0 0 0 0	Lysis†	4-15
17	F 32	66	48	14	66 88 8 8	0 0 0 0	4.2 6.7 Tr 1.8	0 0 0 10 ²	0 0 0 2	Crisis	73
18	M 47	77	40	11	77 122 8 11 19	0 0 0 0 0	4.7 5.4 Tr	0 0 10 ² 10 ² 10 ²	0 0 32 8	Lysis	84-116
19	F 68	8	108	25	8 9 10 13 16	0 0 0 0 0	6.6 6.6 11.4	0 0 0 0 0	0 0 0 0 0	Crisis	9
20	F 43	23	84	22	22 38 5 3 13 15	0 0 0 0 0 0	1.9 1.8 4.9	0 0 0 0 0 0	0 0 0 0 0 0	Crisis	51
21	M 56	?	84	20	-36† 0 +30 +78 +7 +12	0 0 0 0 0 0	7.4 8.0 9.5 9.4	0 0 0 0 10 ² 0	0 0 0 0 0 0	Empty- ema	
22	F 52	7	19	114	7 8 9 11 13 16 22 23 34	0 0 0 0 0 0 0 0 0	1.5 1.3 2.0 4.2 2.9 2.2 4.7 8.2	0 (0) 0 (0) 0 (0) 0 (10 ²) 0 (10 ²) 0 (0) 0 (0) 0 (0) 0 (0)	0 0 0 16 8 0 0 0	Empty- ema	

TABLE IX—Continued

Number	Sex and age	Sulfapyridine therapy			Results of tests						Termination	
		First dose	Interval first to last dose	Amount	Day of disease	Blood culture	Blood sulfa pyridine		Mouse protection*	Agglutinins	Mode	Day
		hours after onset	hours	grams			Frco	Total				
23	M 72	?	108	27	-21 +24 +31 +6 +10 +15 +20 +34	+	5.9	8.7	0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 10 (0) 0 (0) 0 (0)	0 0 0 0 0 0 0 0	Lysis	+40
								mgm. per 100 cc.				
24	F 57	77	48	13	77 121 8 10 14	0 0 0 0 0	4.4	6.1	0 (0) 0 (0) 0 (0) 10 ² (10 ²) 10 ² (10 ²)	0 0 0 4 4	Crisis ? Re-lapse	98 6-18
25	M 65	70	48	12	68 71 103 8 15	0 0 0 Tr Tr	6.6	8.7	10 ² (10 ²) 10 ² (10 ²) 10 (10) - (0) 0 (1)	8 4 4 2 0	Lysis	5-14
26	F 24	110‡	54	16	108 145 10 19	0 0 0 0	8.5	9.0	10 ² 10 ² 10 ² 10 ²	0 2 2 0	Crisis	139
27	F 50	30	72	16	30 53 72 97 9 15	0 0 0 0 0 0	7.9 5.2 2.1	10.1 9.2 4.7	0 0 10 ² 10 ² 10 ² 10 ²	0 0 8 8 16 32	Crisis	68
28	F 39	25¶	37	8	25 37 115 7	0 0 0 0	3.5 5.0 4.3	4.5 8.8 5.8	0 10 ² 10 ²	0 32 8 4	Crisis	51

* Numbers in parentheses represent protection with patient's own pneumococcus

† Vomiting and irrational on 6th to 9th day

‡ From beginning of treatment

§ Otitis media (Pn III) before treatment

|| Blood 2 hours before 60,000 units of serum given in 5 hours.

¶ Serum started at same time, 320,000 units given in 6 hours.

and on patients treated with sulfanilamide with or without serum (9), the antibody response to Type III pneumococcus pneumonia is considerably more variable than in other types. The same variability of response has been found in the cases treated with sulfapyridine. In Tables VIII and IX are presented the results of the various antibody determinations in 28 of the cases due to this type that were studied. These cases will serve to illustrate the variety of the responses noted. The findings in the 14 cases on whose blood bactericidal and opsonic tests were carried out are shown in Table VIII. They include 4

patients treated with serum in addition to sulfapyridine. One-half of the cases tested had bactericidal activity in the blood before treatment was begun. In 2 of them (Numbers 8, 9) the early pneumococcal action was associated with mouse protection and in 1 there were agglutinins demonstrated at the same time.

The findings in all of the cases were similar in most respects to those previously reported in cases of Type III pneumococcus pneumonia when sulfanilamide was used instead of sulfapyridine. However, there was one significant difference. In the bloods obtained following sulfanilamide administration, but before the development of heat-stable antibodies (protection agglutinins, opsonins), there was marked bacteriostasis but little or no bactericidal action was demonstrable. After sulfapyridine therapy on the other hand high degrees of killing occurred when the concentration of the drug was high and moderate numbers of pneumococci were killed in the bloods which had low concentration of sulfapyridine.

A few findings of interest in some of the cases may be mentioned. In Case 4 pneumococcus killing was absent before treatment. This property was present during the administration of sulfapyridine and persisted after treatment when the drug could no longer be demonstrated in the circulation and in spite of the fact that no other antibodies were demonstrable. In Cases 5 and 6 pneumococcal action was no longer demonstrable after the sulfapyridine was discontinued. In Case 6 there were no preliminary tests done before treatment was begun. However the blood culture taken at the time of the first dose of sulfapyridine was sterile and the second one taken 10 hours later was positive for Type III pneumococcus. In this case nitrogen retention and edema developed following sulfapyridine treatment and after the pneumococcal infection had apparently subsided completely. Death occurred after several weeks and resulted for the most part from this complication. In Case 10 observations continued for 4 weeks during which time no agglutinins or protection developed and the titer of pneumococcus killing dropped below its original level after the sulfapyridine was discontinued.

Case 9 is of special interest since in this case there was a recurrence of Type III pneumococcal pneumonia after an interval of 6 months. The

blood culture was positive during the first attack but blood taken at this time and before treatment showed considerable pneumococcal action and some mouse protection. High titer of protective antibody and agglutinins developed after the symptoms subsided. The patient was not seen during the second attack until the middle of the third day of illness. X rays which after the first attack had shown the lesion in the lung to have cleared, again showed pneumonic consolidation. The sputum was rusty and showed Type III pneumococci in pure culture but the blood culture taken at this time was sterile. Studies of the blood at the same time showed only a minimum of pneumococcal action and no agglutinins or protection. None of these antibodies developed during the next 3 days although the symptoms subsided rapidly and completely and the patient left the hospital shortly thereafter.

Among the 28 cases listed in Tables VIII and IX, no protective antibodies could be demonstrated in any of the tests in 6 cases and protection against 10 fatal doses developed in 4 others. Agglutinins were not demonstrated in 8 cases and only minimum titers developed in 4 others. Protective antibody occurred on or before the sixth day just as often in bloods taken before treatment as in those obtained 24 hours or more after the beginning of sulfapyridine therapy. Agglutinins usually developed on the sixth day or later and this was true regardless of whether or not sulfapyridine had previously been instituted.

In 5 patients protection tests were carried out on the same serums with both the stock and the homologous strain of Type III pneumococcus.¹ In 4 of these cases (Numbers 5, 23, 24, 25) the results were in essential agreement. In 1 case however protective antibodies were demonstrated on 2 occasions with the homologous strain but not with the stock strain. Agglutinins, on the other hand were demonstrated with the stock strain in both of these blood samples.

Three hour tests

In a number of cases in which bactericidal tests were done plates were poured from duplicate

¹ The homologous strains were passed through mice daily for 2 or more weeks and were of maximum virulence at the time of the tests.

tubes of blood inoculated with various numbers of organisms after 3 hours' rotation in the incubator. The results in a representative group of cases,

TABLE X

Results of "three-hour pneumococcal tests" in patients with pneumococcus Type III and Type V pneumonia treated with sulfapyridine with or without specific immune serum, and a comparison with other antibodies

Number*	Day of disease	Level of free sulfapyridine	Growth inhibition		Pneumococci killed at 48 hours	Opsonic index	Mouse protection	Agglutinins	Results of 3 hour test			
			24 hours	48 hours					Number of colonies from original inoculum of			
									10 ⁴	10 ³	10 ²	10 ¹

TYPE III CASES												
1	116		0	0	0	0.16	0	0			∞	680
	140	11.8	10 ³	10 ³	10 ⁴	0.02	0	0			4000	0
	168	10.5	10 ³	10 ³	10 ⁴	0	0	0			0	0
	10	9.1	10 ⁴	10 ⁴	10 ⁴	1.4	10 ²	32			2	0
2	14		10 ⁴	10 ⁴	10 ⁴	++	10 ¹	16			1	0
	116		0	0	0	0.9	0	0				1500
	137	6.2	10 ³	10 ⁴	10 ⁴	0.5	0	0				800
	161	8.0	10 ³	10 ⁴	10 ⁴	0.5	0	0				2000
3	10		10 ⁴	10 ⁴	10 ⁴	0.5	10	0				0
	16		10 ⁴	10 ⁴	10 ⁴	0	10	2				0
	108		0	0	0	0	0	0				2000
	120	4.5	10 ³	10 ³	0	0.02	0	0				1600
4	8	9.0	10 ⁴	10 ⁴	10 ⁴	0.02	0	0			0	0
	13	4.3	10 ⁴	10 ⁴	10 ⁴	0.32	10	4			6	0
	58		0	0	0	0.02	0	0				1200
	71	4.3	10 ³	10 ³	10 ³	0	0	0			8	61
5	120	9.5	10 ⁴	10 ⁴	10 ⁴	0	0	0			87	0
	8	Tr	10 ⁴	10 ⁴	10 ³	0.28	0	2			22	0
	12		10 ⁴	10 ⁴	10 ³	0.32	0	0				0
	89		0	0	0	0.22	0	0				400
8	98	3.2	10 ⁴	10 ⁴	0	0.32	0	0				95
	123	5.9	10 ⁴	10 ⁴	10 ³	0.20	0	0				61
	11	0	0	0	0	0	0	0				400
	35		10 ⁴	10 ⁴	10 ⁴	0.46	10 ²	2			0	0
9a	57	1.5	10 ⁴	10 ⁴	10 ⁴	0.16	0	2			5	0
	7	0	10 ⁴	10 ⁴	10 ⁴	5.1	10 ⁴	16			0	0
	65		10	10	10	0.16	0	0				36
	142	2.9	10 ⁴	10 ⁴	10	0.26	0	0			8	7
10	29		10 ⁴	10 ⁴	10 ³	0.26	0	0	1200		0	0
14	30		10 ³	10 ³	10 ³	0.10	0	0				0
	51	7.9	10 ⁴	10 ⁴	10 ⁴	0.06	0	0			∞	0
	9		10 ⁴	10 ⁴	10 ³	+	10 ³	16			2000	5
	16		10 ⁴	10 ⁴	10 ³	+	10 ³	32			4000	79

TYPE V CASES

2	74		10 ⁴	10 ⁴	10 ⁴	0.74	0	0				8
	135	3.5	10 ⁴	10 ⁴	10 ⁴	36.4	0	0				0
3	96		10 ⁴	10 ⁴	10 ⁴	1.58	10	0				0
	107	4.5	10 ⁷	10 ⁷	10 ⁷	1.34	0	0				0
4	86		10 ⁴	10 ⁴	10 ⁴	0.96	10 ⁴	0				0
	96	5.3	10 ⁴	10 ⁴	10 ⁴	0.60	0	0				0
6	120	5.6	10 ⁴	10 ⁴	10 ⁴	0.80	10 ⁴	2				0
	20		0	0	0	1.5	0	0				0
6	43	5.0	10 ⁴	10 ⁴	10 ⁴	0.28	0	0				0
	65	Tr	10 ⁴	10 ⁴	10 ⁴	0.70	10	0				0
	88		10 ⁴	10 ⁴	10 ⁴	15.4	10 ⁴	16				0
	10	0	10 ⁴	10 ⁴	10 ⁴	+	10 ⁴	8				0

* Case numbers are same as in Tables IV and VIII which have the relevant data concerning treatment and outcome.

9 of Type III and 4 of Type V, are listed in Table X. These results show clearly that, during the course of treatment, pneumococcus killing due to the action of sulfapyridine and that due to the immune state may be differentiated by the results of the tests. Where there is marked bacteriostasis early and only slight killing after 48 hours the effect is probably due wholly or in part to sulfapyridine. Where there is killing in 3 hours this is due to the immune properties of the serum, these being either acquired spontaneously or introduced passively. The mechanism of the pneumococcal action when killing of large numbers of pneumococci occurs can be readily differentiated in this manner.

These findings emphasize again the fact that the destruction of pneumococci in the presence of immune bodies is rapid, while such killing when it is due to the presence of sulfapyridine is considerably delayed.

Agglutinin response in sulfapyridine-treated cases of pneumonia associated with other types of pneumococci

The antibody response of patients with pneumonia due to types of pneumococci other than those already dealt with who were treated without specific serums or chemicals has been reported previously (5). The results of tests for agglutinins in sulfapyridine-treated cases of pneumonia due to pneumococci other than Type I, II, III, V, VII, or VIII are listed in Table XI. Patients who received specific serums in addition are omitted from this table. In general the results, as far as can be ascertained from the small number of cases, did not differ materially from those formerly obtained in the untreated cases. A few findings of interest may be noted. Homologous type-specific agglutinins developed in only 21 of the cases. Two of the bacteremic cases who recovered, Numbers 13 and 39, were among the ones in whom agglutinins were not demonstrated although the last tests in these cases were done on the thirteenth and sixteenth day respectively.

Among the patients who developed agglutinins these antibodies were first demonstrated on the fifth day in 3 cases and on the seventh day or later in the others. In 2 of the cases who developed the antibodies early (Numbers 12 and 14) mini-

TABLE XI

Agglutinins for homologous type of pneumococcus in sulfapyridine treated patients with pneumonia due to pneumococci of types other than I, II, III, V, VII or VIII

Number	Sex and age	Type	Treatment		Termination		Day of test	Blood culture	Agglutinin titer	Number	Sex and age	Type	Treatment		Termination		Day of test	Blood culture	Agglutinin titer
					M/ode	Day									M/ode	Day			
1	M 29	IV	5	5	Crisis	5	5 8	0	0 8	23	M 16	XIV	5-8	15	Crisis	6	5 6 9 12 21	0 0 0 0 0	0 8 0 0 0
2	M 44	IV	10- 14	24	Crisis	12	10 11 12 13 17 20	0 0 0 16 4 4	0 0 0 16 4 4	24	M 24	XIV	3-7	23	Crisis	5	3 5 6 7 9 14	0 0 0 0 0 0	0 0 0 0 2 8 4
3	F 74	IV	6	5	Crisis	7	4 7	0 0	0 2										
4	F 32	IV	4-8	22	Crisis	5	4 5 6 7 8 11	+ 0 0 0 0 16	0 0 0 0 0 16	25	F 60	XIV	9- 18	42	Crisis	10	9 11 13 15 25	3 0 0 0 16 32	0 8 8 0 16 32
5	M 35	VI	2-3	9	Died	3	2 3	0 0	0 0	26	M 66	XV	9- 19	60	Died	21	9 11 18	0 0 0	0 0 0
6	F 15	VI	6-9	14	Lysis	7+	3 6 8 17	0 0 0 0	0 0 0 0	27	M 42	XVI	8- 11	18	Crisis*	9	6 7 9 11 13 15 20	0 0 0 0 0 0 0	0 0 4 2 4 0 0
7	F 46	VI	11- 18	42	Lysis	12+	7 11 16 21	0 0 0 0	0 4 2 0	28	M 70	XVII	4-8	22	Crisis	4	3 4 6 8 11	0 0 0 0 0	0 0 0 0 0
8	M 56	IX	7- 11	22	Crisis	9	7 11	0 0	4 4										
9	F 59	IX	7- 10	16	Crisis	8	6 7 8 11	0 0 0 0	0 0 0 0	29	M 65	XVII	3-6	15	Crisis	4	3 4 6 8	0 0 0 0	0 0 0 0
10	F 51	IX	5-7	9	Lysis	5+	6 12 17 25	0 0 0 0	0 0 0 0	30	F 66	XVIII	11- 17	28	Lysis	12	11 12 15	0 0 0	0 2 8
11	M 17	IX	4- 10	31	Lysis	8	1 3 5 8 9 15	0 0 0 0 4 4	0 0 0 0 4 4	31	M 37	XVIII	5- 10	25	Crisis	6	4 6 10	0 0 0	0 0 0
12	M 70	IX	4- 13	38	Lysis	±15	4 5 9 13 21	0 0 0 4 4	0 2 4 4 4	33	F 63	XVIII	7-19 -35	50	Lysis*	31	17 19 20 22 23	+ 0 0 0 0	0 0 0 4 0

TABLE XI—Continued

Number	Sex and age	Type	Treatment		Termination		Day of test	Blood culture	Agglutinin titer	Number	Sex and age	Type	Treatment		Termination		Day of test	Blood culture	Agglutinin titer
					Mode	Day									Mode	Day			
13	M 52	X	days 4-12	grams 42	Crisis	7	4 5 8 13	+ 0 0 0	0 0 0 0	34	F 53	XIX	days 4-9	grams 26	Crisis	8	4 6 9 11 16	0 0 0 0 0	0 0 0 0 0
14	M 62	XI	3-7	19	Crisis	3	3 5 10	0 2 0	0 0 0	35	F 31	XIX	7-9	11	Crisis	9	7 9 12	0 0 0	0 0 0
15	F 19	XI	4-5	8	Crisis	5	4 5 9	0 0 8	0 0 0	36	M 34	XIX	4-6	13	Crisis	6	4 6 9 12	0 0 0 0	0 0 0 0
16	M 37	XI ^b	7-11	26	Died	13	4 8 11	0 0 0	0 4 2	37	M 44	XX	5-7	12	Crisis	6	3 5 8 11 14	+ + + + +	0 0 8 16 16
17	F 80	XI	2-8	24	Lysis	3	2 8	0 0	0 0	38	M 59	XX	4-10	32	Lysis	6+	4 6 10 15 19	0 0 0 0 0	0 0 0 0 0
18	M 32	XII	6-10	19	Crisis	7	5 7 10 15	0 0 0 0	0 0 0 0	39	F 65	XXI	5-7	10	Crisis	6	4 5 6 7 9 12 16	+ 0 0 0 0 0 0	0 0 0 0 0 0 0
19	F 64	XIV	3-7	24	Died	7	3 4 5	240 + 0	0 0 0	40	M 63	XXIX	7-10	41	Lysis (empyema)	11	3 5 8	0 0 0	4 8 2
20	M 74	XIV	28-34	53	Died	34	28 30 ^c 31 34	303 0 0 0	0 0 0 0	41	F 13	XXXI	5-7	11	Crisis	5	4 6	0 0	0 4
21	F 17	XIV	5-6	7	Crisis	5	3 5 8	0 0 8	0 8 8										
22	M 57	XIV	6-11	28	Crisis	7	6 8 9 11 15	0 0 0 0 0	0 0 0 0 0										

^a *Streptococcus hemolyticus* in same and subsequent sputa

^b Sputum positive for tubercle bacilli

^c Meningitis demonstrated on this day

^d Agglutinins for Type V pneumococcus (1-8) in this and later specimens None in previous ones All cases negative for 8 other types

^e Fever 12th to 21st day

^f Pleural fluid culture Pn XVIII on 7th and 11th day

^g Agglutinins Pn I = 2, previous sera negative

^h Three bouts of fever, no agglutinins after 23d day (7 tests)

mal titers were found, and in the third (Case 21) the antibodies were first demonstrated just before sulfapyridine treatment was undertaken. In Case 16 death occurred after agglutinins had developed.

Tests for heterologous antibodies were carried out with one or more types of pneumococci in

every case and with several of the frequent types in the serums of all patients who failed to show antibodies for the homologous type. Agglutinins for heterologous types were demonstrated in only 2 cases. In Case 22 Type XIV pneumococci were obtained from the sputum, homologous agglutinins could not be demonstrated but serums

obtained on the ninth day and later showed agglutinins for Type V pneumococci. Such agglutinins were not present in 2 earlier serums. In Case 32 a low titer of Type I agglutinins was found in the last serum tested.

Two of the patients had other organisms in their sputa which were probably significant with regard to the pulmonary infection. Hemolytic streptococci were found to predominate in all sputa examined in Case 10 and agglutinins for the Type IX pneumococcus which was found in the earlier examination of his sputum were not demonstrated in any of these serums. However in Case 16, the sputum was strongly positive for tubercle bacilli and also contained Type XI pneumococci. Antibodies for this type developed during the disease but the patient died after a few days.

DISCUSSION

When two agents of proven efficacy such as type-specific antipneumococcus serum and sulfapyridine are available for the treatment of any severe or highly fatal disease such as pneumonia, it is most important to determine the relative efficacy of each agent under various conditions. It is equally important to determine whether such agents are more effective when used together than when used separately. While the final test under such circumstances rests in the careful analysis of the response of a large number of cases to therapy a close study of patients under treatment will reveal many of the factors involved in the action of each of these agents when used separately or in combination. Furthermore, while much can be learned from controlled studies of experimental infections in animals the conditions in patients, particularly in cases of pneumonia can hardly be duplicated in animals. It is for that reason that the present studies were undertaken to supplement clinical studies in an attempt to elucidate some aspects of the problems of specific serotherapy and chemotherapy in the pneumococcal pneumonias. It has been shown previously (10) and corroborated in the present studies that the blood of patients with pneumonia may exert bactericidal action against the infecting organism during the course of the disease. This bactericidal action is independent of heat stable antibodies (agglutinins and mouse protection)

In patients lacking this bactericidal action the blood acquires the ability to kill large numbers of the homologous pneumococcus at the time of recovery and in that event the pneumococidal action of the blood is usually associated with agglutinins, mouse protective antibodies and opsonins. These latter heat stable antibodies are also acquired at the time of crisis or later by those patients who previously had pneumococidal power in the blood. The heat stable antibodies may be introduced passively in the course of the disease and may thus bring about an artificial crisis. This has been repeatedly demonstrated in the clinical use of specific antipneumococcal serums.

Recent studies (11) which have been confirmed and extended in this laboratory (1) have shown that sulfapyridine exerts marked bacteriostatic and bactericidal effects on pneumococci in artificial media or when added to human blood *in vitro*. The results of the present studies indicate that blood taken from patients during sulfapyridine therapy has bacteriostatic and bactericidal properties which parallel those found when comparative amounts of sulfapyridine are added to the blood *in vitro*. This pneumococidal action of sulfapyridine has been found to be independent of the immune mechanism when the drug is added to blood *in vitro* and the same is here shown to be true in the blood of patients under treatment. In both the *in vitro* experiments and in the present studies with blood taken during treatment it was shown that the effect of the combination of serum and sulfapyridine is greater than when either agent is used alone.

The results of the 3 hour test are of special interest. It was shown previously (1) that the pneumococidal action of sulfapyridine is a delayed action. It depends on the growth of pneumococci. The fewer the number of organisms present the more rapid the more certain and the more effective is the action of this drug. The pneumococidal properties associated with the immune mechanism whether in natural antibodies such as are found in normal serum or in acquired antibodies resulting from infection or in antibodies passively introduced in therapy all act rapidly. The action due to the immune mechanism is carried essentially to completion during the 'growth phase' of sulfapyridine. (1)

These findings would indicate very strongly that serum and sulfapyridine, at least with respect to their mode of action, are important complementary weapons in combatting the pneumococcic pneumonias. Whether they are both actually necessary, and the conditions under which each or both may be most effective clinically, are not within the scope of the present discussion.

Aside from the importance of the mechanism of action, this paper is concerned largely with another phase of the use of sulfapyridine. It is obviously important to know whether the immune mechanism is essential for recovery when a bactericidal drug like sulfapyridine is used. Only an indirect answer can be given to this question. The data presented indicate that, when specific antibodies are present, recovery is more rapid. They show further that recovery following sulfapyridine therapy is associated with the development of antibodies in the same manner as in the course of natural recovery without the use of the drug. The antibodies develop apparently with the same frequency as in cases not treated with the drug. It is not possible on the basis of the present data to determine with statistical accuracy whether or not the time of the development of antibodies is shortened under sulfapyridine therapy as compared with the natural development of such antibodies in the course of infection. If there is any difference it is obviously not very striking and does not appear on the surface.

One problem directly faced by the clinician is whether the discontinuance of sulfapyridine treatment before the time of the development of antibodies has any influence on the further course of the illness and whether the occurrence of relapses is thereby encouraged. Relapses have been noted during the course of sulfapyridine therapy and particularly when the drug has been discontinued early. These relapses have probably occurred with more than the expected frequency. If the findings here are of any significance in this respect they would indicate that such relapses might be averted by the early use of immune serum to supplement the drug.

SUMMARY AND CONCLUSIONS

The results of immune studies in patients with pneumococcic pneumonia treated with sulfapyri-

dine are presented. A small number of cases treated with specific serums in addition are also included. Bactericidal and phagocytic tests were carried out in many of the cases associated with Types I, III, and V pneumococci.

The results of the bactericidal tests indicated that the blood of patients undergoing treatment with sulfapyridine has marked bacteriostatic and considerable bactericidal action on the homologous type of pneumococcus. This effect was independent of the immune mechanism residing in the blood. This action of sulfapyridine was the same as that noted when comparable concentrations of the drug are added to artificial media or to human blood *in vitro*. The greatest and most rapid bactericidal activity occurred in the presence of heat-stable antibodies (agglutinins, protection, opsonins).

The results of tests for bactericidal action at the end of 3 hours indicate that the pneumococidal action resulting from the immune mechanism is exerted rapidly and is practically carried to completion before any bacteriostatic effect of sulfapyridine becomes evident.

The antibody response of patients with pneumococcic pneumonia treated with sulfapyridine, as far as could be determined was comparable in every respect to that resulting from spontaneous recovery. Protective antibodies rarely developed before the sixth day and agglutinins rarely appeared before the seventh day of the disease.

Entirely apart from observed clinical results and only from the point of view of the mechanism of action of serum and sulfapyridine as observed in patients undergoing treatment, the combination of these two agents is the treatment of choice.

This study was carried out with the technical assistance of Mildred W. Barnes and Claire Wilcox. The chemical determinations were made by Margaret A. Adams and Nancy E. Marean.

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A COMPARATIVE STUDY OF THE BLOOD CONCENTRATIONS AND URINARY EXCRETION OF SULFAPYRIDINE AND SULFANILAMIDE AFTER SINGLE DOSES OF SULFAPYRIDINE AND RELATED COMPOUNDS ADMINISTERED BY VARIOUS ROUTES

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We have recently had available a solution containing a high concentration of sulfapyridine in 50 per cent glucose which can be conveniently administered orally subcutaneously and intravenously (1). This offered an opportunity to study the effect of the route of administration of a drug containing sulfapyridine on the blood level and urinary excretion and to compare it with other derivatives of sulfanilamide. The results of the biochemical studies are presented in this paper. The bacteriological and clinical results are presented elsewhere (1, 2).

METHODS AND MATERIALS

Four subjects were studied. They were maintained on a house diet and their average fluid intake was about 3 liters daily. There was no demonstrable kidney damage present in any of these patients. In the case of Subject B who is reported in detail, the phenolsulfonphthalein excretion was normal and showed no change after the investigation was completed. In no instance did vomiting occur following the administration of any of the drugs investigated nor was there any diarrhoea during the period of observation. Subject A was a 26-year-old white man weighing 127 pounds (58 kgm.) suffering from gonococcal arthritis. Subject B was a 43-year-old white male, weighing 152 pounds (69 kgm.) who had arthritis thought to be of gonococcal origin. Subject C was a 62-year-old white male, weighing 150 pounds (68 kgm.) who was under treatment for a parotid abscess which followed facial erysipelas. Subject D was a 39-year-old white male weighing 156 pounds (70 kgm.) who had active gonococcal arthritis.

The glucose-sulfapyridine solution used in this study was prepared for us by the Research Division of the Lederle Laboratories. It contained 9.5 per cent total

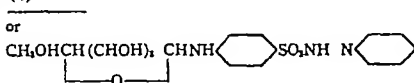
sulfapyridine in 50 per cent glucose solution. Approximately 97 per cent of the sulfapyridine was reported to be in the form of a glucose-sulfapyridine compound, as determined by a direct nitrous acid method. Hydrolysis of the compound takes place rapidly in the presence of cold dilute acids. For this reason the colorimetric method of Marshall and Litchfield (3) cannot be used to determine the amount of sulfapyridine in combination with glucose either in the original solution or in the blood.

During this investigation the glucose-sulfapyridine solution and sulfanilamide were administered intravenously subcutaneously and orally. Sodium sulfapyridine was given intravenously and sulfapyridine was given orally. Each drug was given in 4.75 gram amounts in 500 ml. of fluid. Physiological saline was used for the parenteral injections and tap water for the oral doses. The intravenous injections were given over a period of an hour. The subcutaneous ones in about 1½ hours, and the oral doses were divided into 6 parts and given over a period of an hour. Some of the studies were repeated in individual subjects.

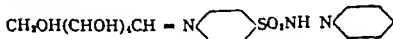
All urine samples were saved. Specimens were obtained simultaneously with the blood samples as far as possible. Determination of sulfapyridine or sulfanilamide was made on all samples. Collection of urine was continued until it showed no appreciable amounts of the drug. Before commencing a study of a second drug or changing the route of administration, a further period of 24 hours was usually allowed to elapse.

In vitro studies were conducted by adding sufficient amounts of the compound under investigation to oxalated whole blood to give concentrations of the substance between 8 and 10 mgm. per 100 ml. The hematocrit was determined in each instance and corrected for the oxalate effect. Samples were removed at various times and the concentration of the drug in the whole blood and plasma was determined.

Determinations of sulfapyridine or sulfanilamide were made by Marshall and Litchfield's method (3) and glucose determinations by the method of Folin and Wu (4).



¹We are indebted to the Research Division of the Lederle Laboratories for assays on the drug. They report the glucose-sulfapyridine compound to be a glucose anil of the general formula



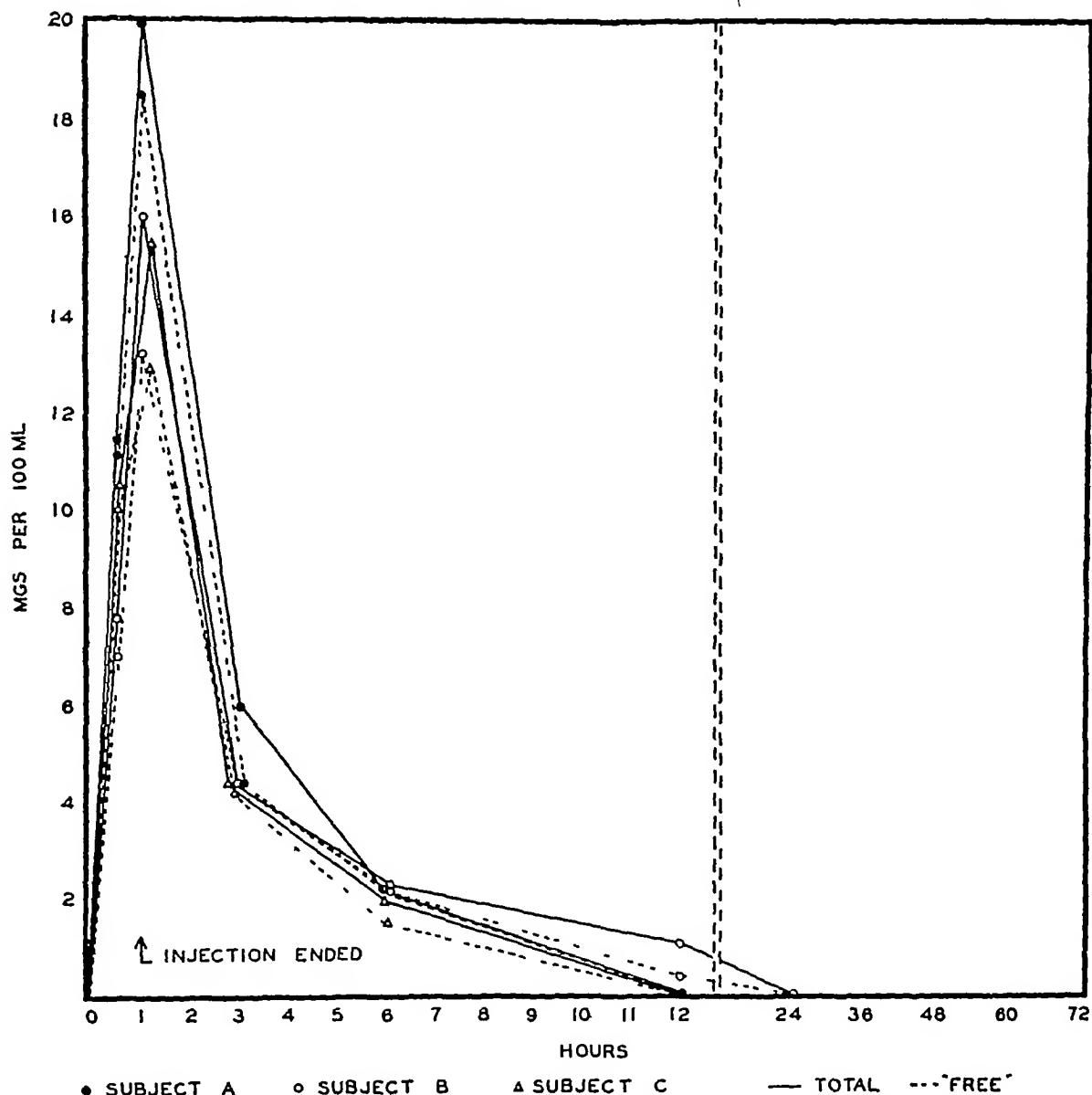


FIG 1 EFFECT OF INTRAVENOUS INJECTION OF GLUCOSE SULFAPYRIDINE ON THE BLOOD LEVEL

EXPERIMENTAL

The effect of the intravenous administration of glucose sulfapyridine, sodium sulfapyridine and sulfamylamide on the blood level and urinary excretion of sulfapyridine and sulfamylamide, respectively

The sulfapyridine concentrations of the blood in three subjects injected with glucose sulfapyridine intravenously are shown in Figure 1. The data for subject B, who was given all the drugs by all the routes used in this study, are shown in

Table I. (This subject's excretion differed in some respects from the other subjects studied in spite of good phenolsulphonphthalein excretion.) During the period of administration the blood level of sulfapyridine rose rapidly, reaching levels between 15 and 20 mgm per 100 ml of blood at the end of the injection. The fall in concentration of the drug was equally rapid, only traces being present in the circulating blood 11 hours after the injection ended.

There was a rise in blood glucose comparable to that found after the intravenous injection of an

TABLE I—Continued

Compound	Route	Time*	Blood			Urine								Remarks
			Concentration of drug			Vol ume	Water excre- tion	Concentra- tion of drug		Excretion of drug		Per cent of administered dose excreted (cumulative)		
			Free†	Total	Con- ju- gated			Free	Total	Free	Total	Free	Total	
		hours	mgm per 100 ml	per cent	ml	ml per minute	mgm per 100 ml		mgm for period		per cent			
Sulfanilamide	Subcutaneous	1	T	1.9	100	180	4.0	2.8	12.7	5	23	0.1	0.5	Injection ended at 1½ hours All values as sulfanilamide
		1½	1.8	3.1	41.9	20	0.4	10.6	21.6	2	4	0.1	0.6	
		3	3.8	5.0	24.0	190	2.1	24.7	41.7	47	79	1.1	2.3	
		6	9.0	10.6	15.1	450	2.5	53.4	77.0	240	346	6.1	9.6	
		12	4.7	6.7	29.9	1600	4.4	46.9	74.1	750	1186	21.9	34.5	
		24	2.1	3.5	40.0	1160	1.6	43.8	110.0	508	1276	32.6	61.4	
		50	1.2	3.0	60.0	2350	1.5	19.9	52.3	468	1229	42.5	87.3	
		74	T	2.1	100.0	1840	1.2	6.2	30.3	114	558	44.9	99.0	
		92½				3000	2.7	1.0	5.8	30	174	45.5	102.7	
		95	T	T		200	1.3	1.6	12.2	3	24	45.6	103.2	
Glucose Sulfapyridine	Oral	1	T	T		400	6.6	0.8	1.6	3	6	0.1	0.1	Administered over first hour All values as sulfapyridine
		3	T	1.7	100	25	0.2	22.6	59.2	6	15	0.2	0.4	
		6	T	1.8	100	150	0.8	26.6	96.1	40	144	1.0	3.4	
		12	T	1.9	100	700	2.0	10.5	43.5	73	304	2.5	9.8	
		24	1.6	2.9	45	650	0.9	23.5	119.0	153	773	5.7	26.1	
		36	1.5	3.0	50	1450	2.0	16.3	73.7	236	1069	10.7	48.6	
		48	T	2.0	100	720	1.0	20.0	128.2	144	923	13.7	68.0	
		72				2800	2.0	3.1	23.4	87	655	15.5	81.8	
		94				2500	1.9	1.0	2.5	25	62	16.0	83.1	
		96	0	0		15	0.5	1.5	5.9		1			
Sulfanilamide	Oral	6	6.6	7.8	15.4	1600	4.4	27.5	40.0	440	640	9.3	13.5	Administration over 1 hour 1st sample at 6 hours All values as sulfanilamide
		12	4.7	6.9	31.9	800	2.2	52.5	88.8	420	713	18.1	28.5	
		24	3.3	7.1	53.5	1050	1.4	39.2	99.0	412	1040	26.8	50.1	
		36	1.9	3.8	50.0	1200	1.7	9.6	26.4	115	316	29.2	57.1	
		48	1.3	3.2	59.1	1000	1.4	11.8	43.7	118	437	31.7	66.3	
		72	T	2.0	100.0	2700	1.9	5.4	21.8	146	588	34.8	78.7	
		96	T	1.5	100.0	3100	2.2	1.8	6.5	56	201	36.0	82.9	
		120	0	T		2900	2.0	T	3.9		115		85.3	
		141	0	Sl T		2900	2.3	T	1.0		29		85.9	
		144				300	1.7	T	2.1		6		86.0	
Sulfapyridine	Oral	6	4.4	6.5	32.3	1600	4.4	10.8	19.7	173	315	3.6	6.6	Administration over 1 hour 1st sample at 6 hours All values as sulfapyridine
		12	3.2	7.4	56.8	820	2.3	26.4	84.4	216	692	8.1	21.2	
		24	1.4	3.6	61.1	550	0.8	30.2	15.0	166	1182	11.6	46.1	
		48	Sl T	T		2800	1.9	5.2	41.8	146	1170	14.7	70.7	
		72½	0	V Sl T		2950	2.0	1.1	8.6	32	254	15.4	76.0	
		96				2950	2.1	0.5	1.6	15	47	15.7	77.0	
		120				3400	2.4	T	0.6	T	20		77.4	

* All times recorded from commencement of administration of drug

† The values given for "free" sulfapyridine following the administration of *glucose sulfapyridine* refer only to the values given by the Marshall method without hydrolysis (3) and do not imply that the sulfapyridine was present in the free form

T = Trace Sl T = Slight trace V Sl T = Very slight trace

TABLE II

The effect of the intravenous administration of glucose and of glucose sulfapyridine on the concentration of glucose in the blood and urine of Subject B

Time	Glucose		Glucose sulfapyridine	
	Blood glucose	Urine glucose	Blood glucose	Urine glucose
hours	mgm per 100 ml	grams per 100 ml	mgm per 100 ml	grams per 100 ml
Fasting	90.9	Less than 0.10	115.0	0.25
½ hour after injec.	144.5	Less than 0.10	181.1	
1 hour after injec.	171.8	Less than 0.10	227.5	0.20
2 hours after injec.	115.6	Less than 0.10		
3 hours after injec.	96.6	Less than 0.10	95.8	0.20
4 hours after injec.	84.4	Less than 0.10		
6 hours after injec.				Less than 0.10
12 hours after injec.				Less than 0.10

between 40 and 46 per cent was circulating as the conjugated drug

The excretion of sulfapyridine into the urine after the injection of sodium sulfapyridine was much slower than in the case of the glucose compound (Table I). In 96 hours, however, 90 per cent of the administered sulfapyridine had been excreted. About 75 per cent of the total sulfapyridine excreted was in the form of the conjugated drug and this percentage was approximately the same whether the sodium salt or the glucose compound was injected.

The effect of diuresis on the urinary excretion of sulfapyridine was not marked in Subject B, but in the other two individuals the maximum excretion of the drug into the urine corresponded to a period of maximum diuresis. There was no parallelism between the blood level and the ex-

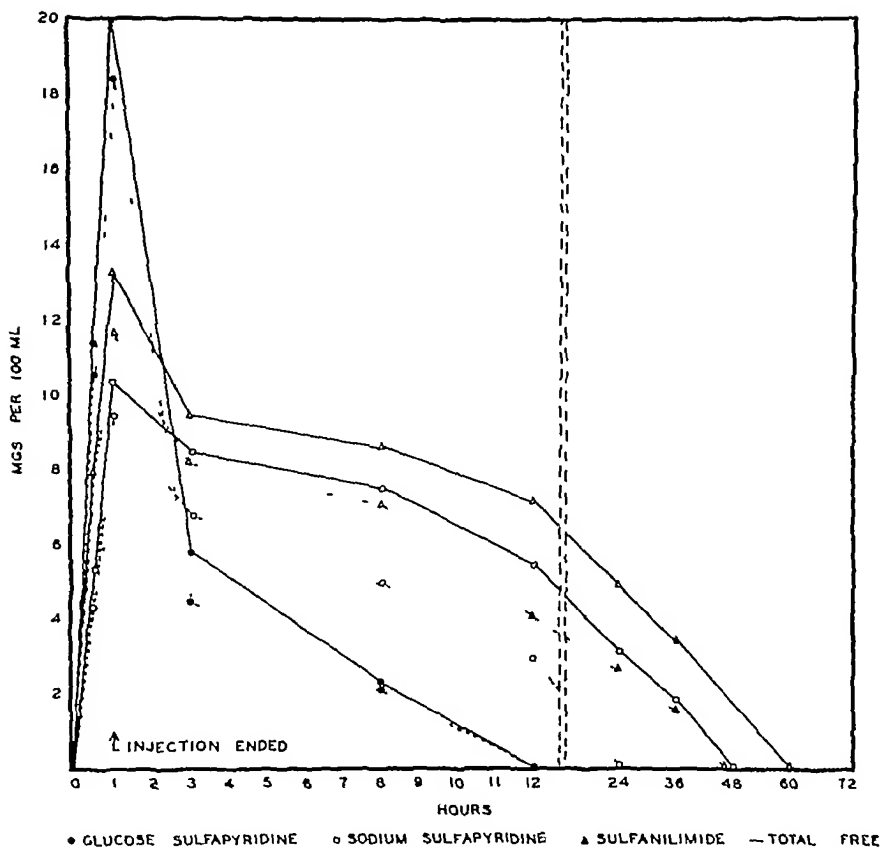


FIG. 2. (SUBJECT B) BLOOD LEVELS AFTER INTRAVENOUS ADMINISTRATION OF THREE SULFANILAMIDE DERIVATIVES

cretion rate of sulfapyridine such as obtained for the glucose compound.

The injection of sulfanilamide gave similar results except that there was a somewhat lower concentration of conjugated sulfanilamide present in the urine than that found after the injection of a corresponding amount of sodium sulfapyridine.

The effect of the subcutaneous administration of glucose sulfapyridine and sulfanilamide on the blood level and urinary excretion of sulfapyridine and sulfanilamide respectively

The blood concentrations of sulfapyridine following the subcutaneous injection of "

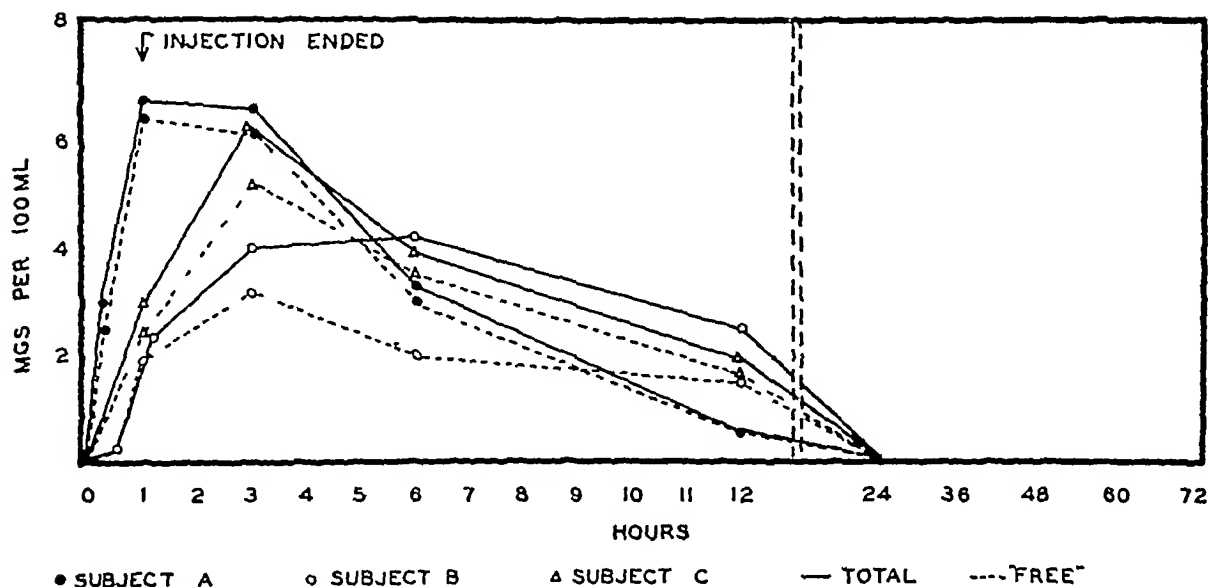


FIG 3 EFFECT OF SUBCUTANEOUS INJECTION OF GLUCOSE SULFAPYRIDINE ON THE BLOOD LEVEL

fapyridine are shown for the three subjects in Figure 3, and the data obtained on a second occasion in Subject B are shown in Table I. The concentration of sulfapyridine in the blood rose more slowly and to a lower maximum than when glucose sulfapyridine was given intravenously. Determinable amounts were present in the circulating blood for at least 12 hours. There was no marked increase in the conjugated form as was the case when the sodium salt or sulfanilamide was given intravenously.

The subcutaneous injection of glucose sulfapyridine was followed by a hyperglycemia comparable to that produced by a similar amount of glucose. There was, however, no corresponding glycosuria. All of the sulfapyridine was recovered in the urine within 24 to 48 hours, from 77 to 87 per cent appearing in the conjugated form.

Following the subcutaneous injection of sulfanilamide, the blood levels rose slowly to a maximum of between 8 to 10 mgm per 100 ml 6 hours after the injection was started. In Subject B (Table I and Figure 4) determinable amounts of free sulfanilamide were present 50 hours after commencement of injection, in a second subject only traces were present at this time. In both instances, however, levels between 2 and 3 mgm of free, and 3 to 5 mgm of total sulfanilamide per 100 ml of blood were present 24 hours after

injection. During the time when the blood levels were high, between 70 and 89 per cent of the drug was present in the free form.

All of the subcutaneously injected material was recovered from the urine in 96 hours, 38 per cent of this was present as the free and 62 per cent as the conjugated drug. The maximum excretion occurred during or shortly after a period of maximum diuresis. The essential difference between the urinary excretion of sulfapyridine after injection of the glucose compound and the excretion of sulfanilamide, when both were given subcutaneously, was the greater rapidity of the removal of glucose sulfapyridine from the blood.

The effect of the oral administration of glucose sulfapyridine, sulfapyridine, and sulfanilamide on the blood levels and urinary excretion of sulfapyridine or sulfanilamide

The blood levels after oral administration of glucose sulfapyridine in three subjects are shown in Figure 5, and the data for Subject B appear in Table I. The absorption from the gastrointestinal tract was very slow. Appreciable concentrations did not appear in the blood until 24 hours after the beginning of ingestion. Unlike the findings after parenteral administration of the drug, there were large amounts of conjugated sulfapyridine present in the circulating blood.

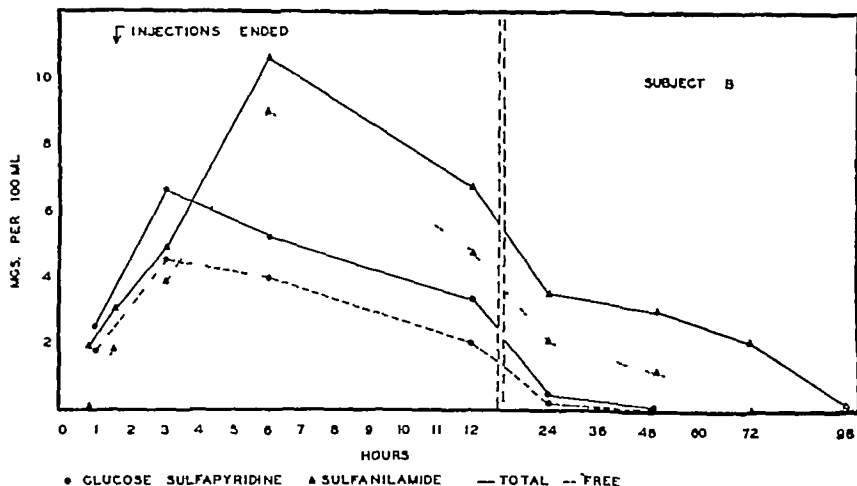


FIG. 4 GLUCOSE SULFAPYRIDINE AND SULFANILAMIDE EFFECT OF SUBCUTANEOUS INJECTION ON THE BLOOD LEVEL

About 80 per cent of the drug administered was recovered from the urine in 72 hours. Approximately 25 per cent of this amount was in the free form. The maximum excretion of the drug occurred during the interval between 24 and 36 hours which corresponded to maximum levels in the blood.

For purposes of further comparison, additional data on the effect of the administration of glucose sulfapyridine by the three different routes on the blood levels of sulfapyridine in Subject A are shown in Figure 6. The data on the recovery of this drug from the urine of the same subject are shown in Figure 7.

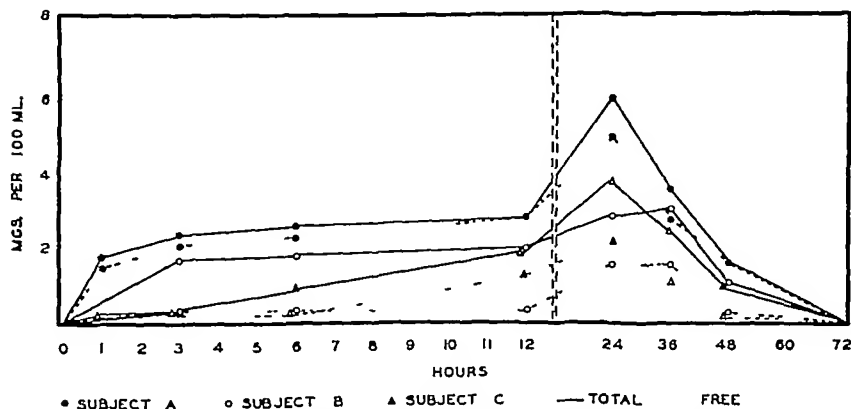


FIG. 5 EFFECT OF ORAL ADMINISTRATION OF GLUCOSE SULFAPYRIDINE ON THE BLOOD LEVEL

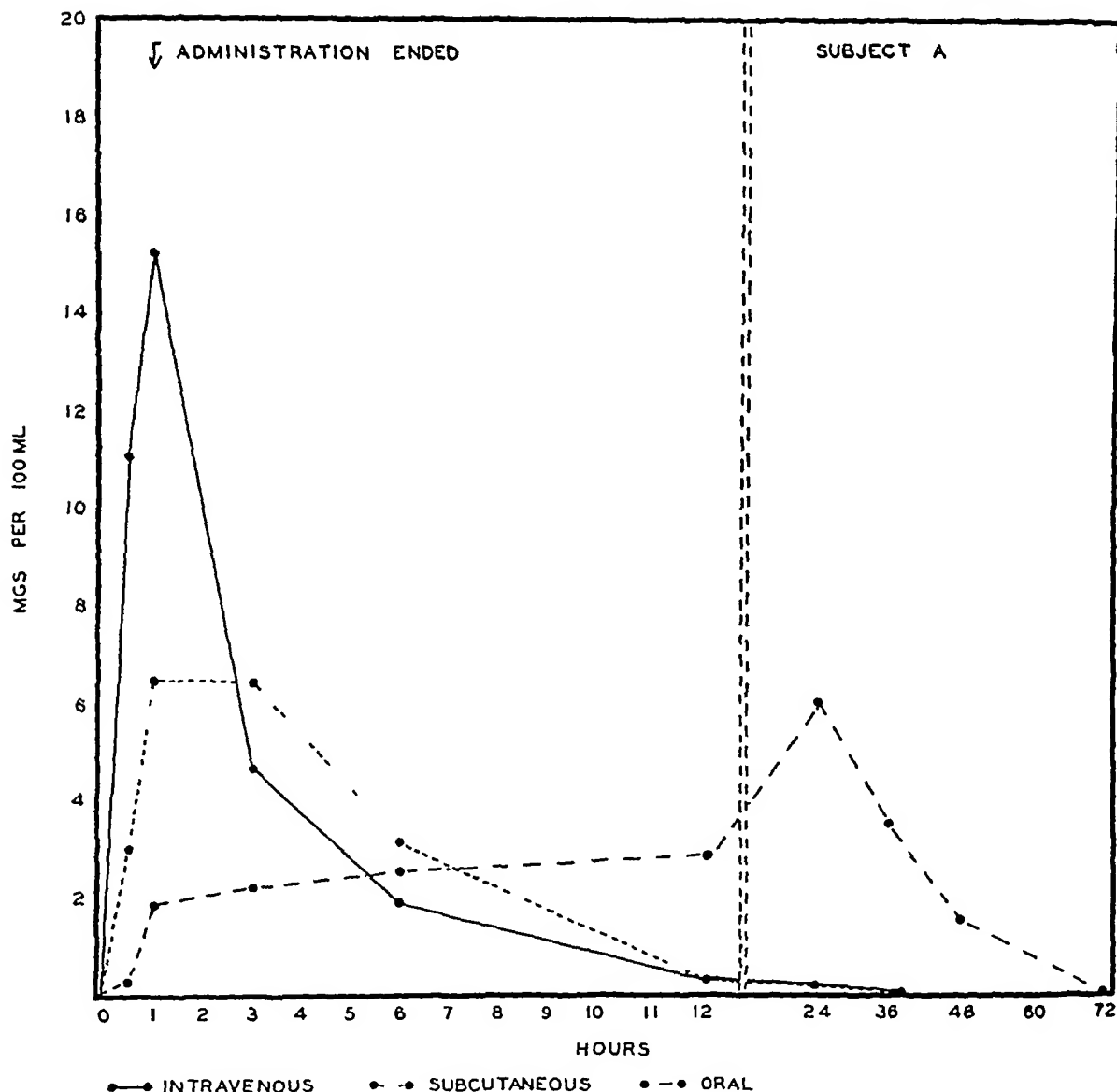


FIG 6 GLUCOSE SULFAPYRIDINE EFFECT OF ROUTE OF ADMINISTRATION ON BLOOD LEVEL

When equivalent amounts of sulfapyridine or sulfanilamide were given orally, the rate of absorption from the gastro-intestinal tract was much faster than for the glucose compound. Values of 4 to 8 mgm of free sulfapyridine or sulfanilamide per 100 ml of blood were obtained 6 hours after the beginning of ingestion and determinable levels were present in the blood 24 hours later. A comparison of the effect of the administration of these two drugs with glucose sulfapyridine is shown in Figure 8 and in Table I. Similar results for the absorption and excretion of sulfapyridine and sul-

fanilamide after oral ingestion have been reported by others (5, 7, 8).

The distribution between red blood cells and plasma of certain derivatives of sulfanilamide in vitro

The fundamental differences in the behavior of glucose sulfapyridine and other sulfanilamide derivatives given intravenously and subcutaneously made appropriate a further consideration of the data in the light of blood clearance and distribution in the body. However, since the investi-

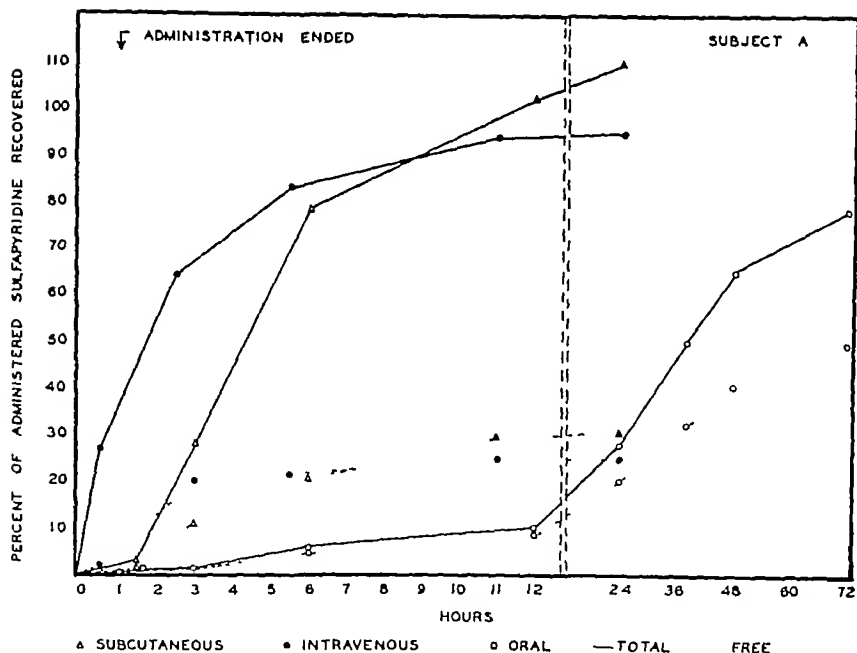


FIG. 7 GLUCOSE SULFAPYRIDINE EFFECT OF ROUTE OF ADMINISTRATION ON URINARY ELIMINATION

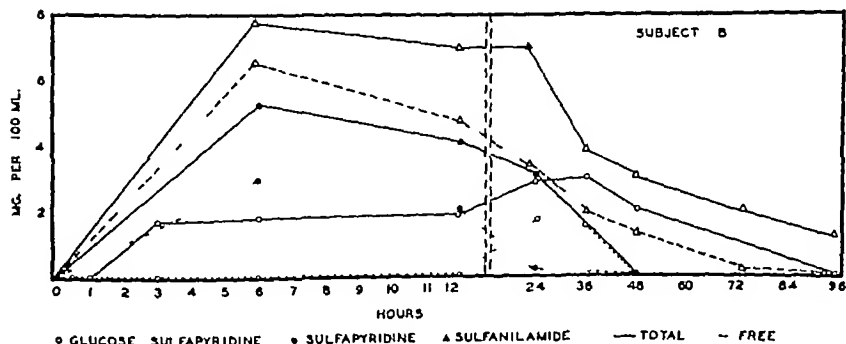


FIG. 8 BLOOD LEVELS AFTER ORAL ADMINISTRATION OF THREE

TABLE III

Distribution between whole blood and plasma of glucose sulfapyridine, sodium sulfapyridine and sulfanilamide in vitro

Subject	Compound given	Time from mixing	Whole blood values*	Plasma values*	Calculated values* in plasma for 100 ml. blood	Calculated values* in cells for 100 ml. blood	Hematocrit (corrected)
T	Glucose Sulfapyridine	10 minutes	mgm. per 100 ml	mgm. per 100 ml			
			9.0	16.4	8.4	0.6	48.8
T	Sodium Sulfapyridine	10 minutes	8.7	9.6	4.9	3.8	48.8
Lo	Glucose Sulfapyridine	10 minutes	11.2	19.8	11.5	0	41.8
Lo	Sodium Sulfapyridine	10 minutes	7.7	8.2	4.8	2.9	41.8
F	Glucose Sulfapyridine	10 minutes	9.1	15.6	8.3	0.8	46.9
F	Glucose Sulfapyridine	1 hour	9.0	16.1	8.5	0.5	46.9
F	Glucose Sulfapyridine	3 hours	8.9	16.0	8.5	0	46.9
F	Sodium Sulfapyridine	10 minutes	8.3	8.7	4.6	3.7	46.9
F	Sodium Sulfapyridine	1 hour	8.2	8.6	4.6	3.6	46.9
F	Sodium Sulfapyridine	3 hours	8.2	8.6	4.6	3.6	46.9
F	Sulfanilamide	10 minutes	9.7	7.7	4.1	5.6	46.9
F	Sulfanilamide	1 hour	9.4	7.4	3.9	5.5	46.9
F	Sulfanilamide	3 hours	9.3	7.5	4.0	5.3	46.9
Low	Glucose Sulfapyridine	10 minutes	9.9	18.9	9.5	0	50.2
Low	Glucose Sulfapyridine	1 hour	9.7	17.6	8.8	0.9	50.2
Low	Glucose Sulfapyridine	3 hours	9.9	18.5	9.3	0.6	50.2
Low	Sodium Sulfapyridine	10 minutes	8.1	8.7	4.4	3.7	50.2
Low	Sodium Sulfapyridine	1 hour	8.1	8.8	4.4	3.7	50.2
Low	Sodium Sulfapyridine	3 hours	8.1	8.2	4.1	4.0	50.2
Low	Sulfanilamide	10 minutes	8.5	7.6	3.8	4.7	50.2
Low	Sulfanilamide	1 hour	9.3	7.5	3.8	5.5	50.2
Low	Sulfanilamide	3 hours	9.7	7.4	3.7	6.0	50.2

* Sulfapyridine or sulfanilamide

gation had been made on whole blood, the distribution of these compounds between red blood cells and plasma was studied

The distribution of glucose sulfapyridine, sodium sulfapyridine and sulfanilamide between red blood cells and plasma was determined *in vitro* at various times up to 3 hours after their addition to oxalated blood. The results are given in Table

III. Glucose sulfapyridine was present only in the plasma, sodium sulfapyridine was equally distributed between the red blood cells and the plasma, and sulfanilamide showed a slightly greater concentration in the red cells than in the plasma. This increased concentration in the red cells of sulfanilamide was pointed out previously by Sise (6). This slight increase in concentration in the red blood cells was disregarded in subsequent calculations.

The distribution of glucose sulfapyridine between red cells and plasma in vivo

Two subjects were given 4.75 grams of glucose sulfapyridine intravenously, two others received the same amount subcutaneously and three were given 12.5 grams of the drug orally. Hematocrits were determined and estimations of sulfapyridine were made on the whole blood and plasma at appropriate intervals. The data are shown in Table IV. Like the *in vitro* results, glucose sulfapyridine showed little penetration into the red blood cells when given intravenously or subcutaneously, while following oral administration approximately one-third of the sulfapyridine found in the whole blood was present in the red cells.

TABLE IV

Distribution of sulfapyridine between red blood cells and plasma when glucose sulfapyridine was administered by various routes

Subject	Route	Time after administration	Whole blood sulfapyridine	Plasma sulfapyridine	Calculated sulfapyridine in plasma for 100 ml. blood	Calculated sulfapyridine in cells for 100 ml. blood	Hematocrit (corrected)
			mgm. in 100 ml.	mgm. in 100 ml.			
I	Intravenous	1 hour	10.8	17.2	10.5	0	38.9
I	Intravenous	3 hours	7.4	10.8	6.6	0.8	38.9
II	Intravenous	1 hour	9.1	16.3	9.3	0	43.3
II	Intravenous	3 hours	4.1	7.1	4.0	0	43.3
III	Subcutaneous	3 hours	3.9	5.5	3.4	0.5	38.6
III	Subcutaneous	4 hours	3.3	4.6	2.8	0.5	38.6
IV	Subcutaneous	3 hours	4.3	7.8	4.4	0	42.8
IV	Subcutaneous	4 hours	3.9	5.6	3.2	0.7	42.8
V	Oral	17 hours	10.5	12.5	6.8	3.7	46.4
VI	Oral	11½ hours	10.6	11.0	6.1	4.5	45.1
VII	Oral	11½ hours	12.0	14.6	8.3	3.7	43.2

The distribution of glucose sulfapyridine in the body water after both intravenous and subcutaneous injection shows that the drug is confined essentially to the extra-cellular water (Table V)

TABLE V
Distribution of glucose sulfapyridine

Patient	Route	Time after beginning of injection in hours	Total sulfapyridine in serum water at end of period	Total sulfapyridine excreted in urine through end of period	Liters of body water	Per cent of body weight
B	Intravenous	3	mgm. 5.4	mgm. 2357	liters 25	per cent 35
A	Intravenous	3	6.4	3024	31	53
O	Intravenous	3	7.4	2346	33	49
B	Subcutaneous	13	4.3	3518	28	33
A	Subcutaneous	6	4.5	3734	28	48

Sodium sulfapyridine, sulfapyridine and sulfanilamide, when given by each of the three routes were distributed over the total body water. Unfortunately variations in the amounts of free and conjugated sulfapyridine or sulfanilamide and secretion of these compounds into the gastrointestinal tract prohibit determination of the distribution. In all instances however the apparent distribution of sulfanilamide and sulfapyridine or its sodium salt was in a volume of water in excess of 70 per cent of the body weight and thus was also true following the oral administration of glucose sulfapyridine.

The renal clearance of sulfanilamide and certain of its derivatives when administered by different routes

The clearance values given in Table VI were obtained by recalculation of the whole blood data on three subjects making use of the distributions between red cells and plasma to obtain serum levels. When given by the intravenous route glucose sulfapyridine differed sharply from either sodium sulfapyridine or sulfanilamide. The latter two substances were cleared at rates indicating a considerable degree of tubular reabsorption while the glucose compound was cleared at a rate usually associated with simple glomerular filtration.

When glucose sulfapyridine and sulfanilamide were given subcutaneously the clearances for the

TABLE VI
Blood clearances of glucose sulfapyridine, sodium sulfapyridine and sulfanilamide when administered by different routes

Patient	Compound	Route	Time of period	Average serum concentration (calculated)		Total drug* excreted in urine for period		Blood cleared per minute		
				Free	Total	Free	Total	Free	Conjugated	Total
B	Glucose Sulfapyridine	Intravenous	120	mgm. per 100 ml. 13.3		mgm. 3325		ml. 135		
A	Glucose Sulfapyridine	Intravenous	150		13.7		1737			114
C	Glucose Sulfapyridine	Intravenous	120		12.0		1718			90
B	Sodium Sulfapyridine	Intravenous	120	7.3	8.5	122	192	14	48	19
A	Sodium Sulfapyridine	Intravenous	150	7.5	7.7	429	508	33		37
C	Sodium Sulfapyridine	Intravenous	120	7.8	10.0	181	256	20	23	21
B	Sulfanilamide	Intravenous	120	10.0	11.5	340	301	20	34	23
D	Sulfanilamide	Intravenous	105	5.7	7.1	478	728	41	83	83
B	Glucose Sulfapyridine	Subcutaneous	360		8.3		1102			49
A	Glucose Sulfapyridine	Subcutaneous	120		9.0		1267			117
O	Glucose Sulfapyridine	Subcutaneous	180		7.2		1714			120
B	Sulfanilamide	Subcutaneous	180	5.3	7.0	240	345	23	49	27
D	Sulfanilamide	Subcutaneous	260	3.1	6.3	643	1067	36	63	43
B	Glucose Sulfapyridine	Oral	720	1.4	2.7	234	1060	23	80	83
A	Glucose Sulfapyridine	Oral	720	3.4	4.3	593	954	23	72	23
O	Glucose Sulfapyridine	Oral	720	1.7	2.9	299	985	24	80	47
B	Sulfapyridine	Oral	720	2.1	4.0	168	1183	11	49	23
A	Sulfapyridine	Oral	720	2.8	4.0	606	804	23		23
C	Sulfapyridine	Oral	360	2.7	3.6	506	1153	38	99	69
B	Sulfanilamide	Oral	720	1.4	2.3	118	437	13	28	19
A	Sulfanilamide	Oral	360	3.3	4.3	606	904	47	111	61
C	Sulfapyridine	Oral	360	4.0	6.7	530	1400	27	142	68

* = Sulfapyridine or sulfanilamide.

† = Determinations were done on serum instead of whole blood

glucose compound were elevated above that for sulfanilamide. With one exception (Subject B) the values for the clearances were found after

When
dine, "

similar values. For all the compounds given orally the clearance values indicated marked tubular reabsorption. The clearance of the conjugated form of sulfanilamide or sulfapyridine was always higher than the free form. The tubules apparently selectively reabsorb the free form of sulfapyridine and sulfanilamide.

DISCUSSION

The data presented show that, when glucose sulfapyridine is given intravenously or subcutaneously, its behavior is quite different from that of the other derivatives of sulfanilamide used in this study when they are given by the same route. The levels of total sulfapyridine in the blood are comparatively higher but these high levels are not maintained. After a brief interval no circulating sulfapyridine is demonstrable.

From the clearance data it is possible to say that intravenous glucose sulfapyridine is eliminated with very little reabsorption while sulfapyridine and its sodium salt, given by parenteral or oral routes, and glucose sulfapyridine, given by mouth, undergo considerable tubular reabsorption. This would indicate that glucose sulfapyridine given intravenously or subcutaneously is not converted to sulfapyridine or sodium sulfapyridine but retains its identity in the blood and is excreted at a different rate. The low clearance values following the oral administration are of the same order of magnitude as those obtaining for sulfapyridine. There is a corresponding increase in the concentration of sulfapyridine in the red blood cells. It would appear, therefore, that orally administered glucose sulfapyridine is largely converted to sulfapyridine or its sodium salt before absorption.

Calculations of the distribution of glucose sulfapyridine given by parenteral route show that the drug is distributed over the extracellular fluid. In this regard it behaves in sharp contrast to sulfapyridine and sodium sulfapyridine which, by approximate calculation, appear to be distributed over the total body water. The distribution of the various compounds between red blood cells and plasma offers still further confirmation of these observations. The oral administration of glucose sulfapyridine differs from that of sulfapyridine and sulfanilamide. The absorption is

slower, maximum concentrations are lower and are found 24 hours or longer after the ingestion of the drug.

These observations are interpreted as meaning that, following intravenous or subcutaneous administration, glucose sulfapyridine circulates in the blood unchanged. However, it would appear that the material present in the urine was not glucose sulfapyridine. This is suggested by the failure to show any glycosuria comparable to the amount of sulfapyridine present in the urine, and also by the presence of a conjugated compound determinable as such by the Marshall and Litchfield method (3).

The results of studies on the pneumococidal activity of glucose sulfapyridine in human blood were in accord with these findings (2). The addition of glucose sulfapyridine to blood in high concentration *in vitro* produced no pneumococidal activity. On prolonged standing at 37° C only slight bacteriostasis became evident. When blood was withdrawn from patients to whom glucose sulfapyridine was given intravenously or subcutaneously, no pneumococidal power was evident. Even should some activity occur in the blood after prolonged standing, the present data show that the drug is removed too quickly from the circulating blood for such a possibility to arise *in vivo* after parenteral administration. Following the oral administration of glucose sulfapyridine the pneumococidal power of the circulating blood was practically the same as that demonstrable with blood having similar concentrations of drug after sulfapyridine itself had been given. These studies offer further evidence that the substance present in the blood after intravenous or subcutaneous administration of glucose sulfapyridine is not free sulfapyridine. They also suggest that the oral administration of glucose sulfapyridine results in its decomposition with the formation of active sulfapyridine.

It is of interest that such a slight procedure as boiling a drug in glucose solution should so change its biochemical and physiological activity. It is suggested that such studies as the ones presented, when applied to new therapeutic substances of this type, may be helpful in determining the potentialities of a drug. Glucose sulfapyridine by these criteria, when given by the

parenteral route has characteristics which indicate that it is probably not useful therapeutically. The rapidity with which it leaves the blood stream, its poor distribution and its apparent failure to hydrolyze into an active substance all militate against its usefulness.

CONCLUSIONS

1 Glucose sulfapyridine administered intravenously or subcutaneously gives high levels of the drug in the blood but is rapidly excreted and appears to circulate in a form which does not behave like sulfapyridine.

2 The data for the clearances of glucose sulfapyridine after its intravenous or subcutaneous administration indicate that little or no reabsorption of glucose sulfapyridine takes place.

3 Glucose sulfapyridine administered orally is absorbed slowly. In other respects, however, the drug present in the blood after its absorption from the gastro-intestinal tract behaves in the same manner as when sulfapyridine itself is given.

4 Glucose sulfapyridine given intravenously or subcutaneously does not enter the red blood cells and is apparently distributed only in the extracellular fluids.

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of Internal Medicine, Yale University Medical School in the interpretation of the clearance and distribution data.

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BACTERICIDAL ACTION OF SODIUM SULFAPYRIDINE AND OF A GLUCOSE-SULFAPYRIDINE SOLUTION IN HUMAN BLOOD

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absorption excretion distribution in the body and renal clearance. A limited clinical trial indicated that while mild cases apparently responded favorably those with typical pneumonia did not seem to show a response consistent with the full activity of the original drug (3).
The present paper deals with studies of the bactericidal properties of human blood to which glucose-sulfapyridine solution was added *in vitro* and similar studies with blood of patients following the administration of this solution by various routes. Comparable studies with sodium sulfapyridine are also presented.

MATERIALS AND METHODS

The pneumococcal tests were carried out as in the other studies mentioned (1) (2) The stock Type III pneumococcus was used. Preliminary tests with this organism in the blood of each of the subjects used in these studies showed either no bactericidal action, or only 10 duplicate were killed in 0.5 cc. of blood. Control tests with these bloods repeated at the time of the *in vivo* experiments and also just prior to the administration of the drugs gave the same results. The glucose-sulfapyridine solution was prepared by the Research Division of the Lederle Laboratories and was furnished in sealed ampules each containing 50 cc. of the sterile solution equivalent to 25 grams of glucose and approximately 5 grams of sulfapyridine. Only freshly opened vials were used in order to minimize the possibility of hydrolysis. The concentrations of sulfapyridine in the blood were determined by the method of Marshall and Litchfield (5). Presumably any sulfapyridine present in chemical combination with glucose is hydrolyzed during the determination of free sulfapyridine by this method. This must be borne in mind whenever values for "free" sulfapyridine are given, since the amounts present in combination with glucose were not determined separately. There was no significant change in the pH of the broth media when sodium sulfapyridine was added in the concentrations used in these tests. In all *in vitro* tests, the drugs were added to 0.5 cc. of blood in a volume of 0.1 cc. of saline containing sufficient amounts to bring the final concentration of sulfapyridine in the blood to 10 mgm. per 100 cc. (1:10,000)

The importance of careful biological control of new chemotherapeutic agents is generally appreciated. It is not always fully appreciated however that similar control may be equally essential when a chemical of known activity and of proven efficacy is used in some new manner. In this paper we wish to present evidence that treatment of a chemical with proven antibacteriostatic activity in a simple manner such as might be used to facilitate its administration may alter its antibacterial properties materially.

In a previous paper (1) we presented data which indicated that sulfapyridine in concentrations usually attainable in therapy has marked bacteriostatic and considerable bactericidal effects on pneumococci grown in favorable artificial media. The same properties were also demonstrated when this drug was added to human blood *in vitro* or when it occurred in the blood of patients under treatment (2). The low solubility of this compound and the desirability of having greater concentrations in solution for parenteral administration led to the use of glucose as a medium to effect such solubility. With 50 per cent glucose in water it was found possible to obtain the complete solution of 25 per cent or more of sulfapyridine, but boiling was necessary to bring this about. Clinical tests indicated that such a solution containing 10 per cent sulfapyridine could be given intravenously and subcutaneously without ill effects provided only that it was diluted to isotonicity with respect to the glucose when given by the latter route (3).
This glucose-sulfapyridine solution was used for detailed studies of the blood concentrations and urinary excretions after administration by various routes and also for comparisons in these respects to sulfapyridine sodium sulfapyridine and sulfanilamide. These studies which are reported separately (4) gave indirect evidence that a new compound was formed which had proper-

RESULTS

Effect of glucose-sulfapyridine and sodium sulfapyridine when added to defibrinated blood in vitro

The results of pneumococcal tests with the blood of 2 subjects are given in Table I. The blood of subject S was used on two occasions, 1 week apart. On both occasions the addition of glucose-sulfapyridine solution in amounts equivalent to 10 mgm per 100 cc (1:10,000) did not

TABLE I

Pneumococcal action of sodium sulfapyridine and of glucose-sulfapyridine when added to defibrinated human blood in vitro

Subject	Material added	Concentration	Growth inhibition		Pneumococcal action at 48 hours	
			24 hours	48 hours	Inoculum	Growth
S	0	10 mgm per 100 cc	0	0		
	G SP		10	0		
S*	0		0	0		
	G SP	10	0	0		
	SP	10	10 ⁵	10 ⁴	10 ⁵	96
	Glucose	50			10 ⁴	0
F	0		10	10	10	10
	G SP	10	0	0		
	NaSP	10	10 ⁴	10 ⁴	10 ⁵	0
	SP	10	10 ⁵	10 ⁵	10 ⁵	1200
F†	0		0	0		
	G SP	10	10 ⁵	0		
	NaSP	10	10 ⁵	10 ⁴	10 ⁴	8
					10 ⁵	8
					10 ⁵	0

* Blood taken 1 week later

† Same blood kept at 37° C for 30 hours. The drugs were added prior to incubation.

Explanation of Tables I and II

Amount. In the case of the glucose-sulfapyridine this represents total amounts of sulfapyridine in the solution.

Concentration. Given in mgm per 100 cc. When added *in vitro*, the final concentration is given, except the glucose, for which the added concentration is given.

Growth inhibition. Numbers represent the largest inoculum which showed no color change.

Pneumococci killed. Listed only when inhibition was noted. Growth = number of colonies grown in agar pour plates. Inoculum refers to original number. Larger inocula yielded too many colonies to count, smaller ones yielded no growth.

Abbreviations. SP = sulfapyridine

G SP = glucose-sulfapyridine solution

NaSP = sodium sulfapyridine

I V = intravenous

SC = subcutaneous

P O = oral

result in any pneumococcal activity. That this lack of activity was not due to the presence of glucose as such was shown by the fact that, when both glucose and sulfapyridine were added in the same proportions as those originally present in the glucose-sulfapyridine solution, there was marked pneumococcal action. The extent of this action was the same as when no glucose was added (1).

The blood of subject F killed 10 diplococci when first tested. The same fresh blood showed no pneumococcal activity when glucose sulfapyridine was added, but considerable numbers were killed and the growth of even larger numbers was inhibited after the addition of sulfapyridine or its sodium salt.

Effect of incubation at 37° C

Duplicate control tests and tests with the addition of glucose sulfapyridine and sodium sulfapyridine were carried out with the blood of subject F after incubation for 30 hours before the organisms were added. There was no bactericidal activity in the blood without the drug. Slight inhibition of growth for 24 hours occurred in the blood containing glucose-sulfapyridine, while inhibition and killing in the blood containing sodium sulfapyridine were essentially the same as in the fresh blood.

The loss of the small amount of pneumococcus killing in the blood of subject F after incubation is consistent with the destruction of complement which is essential for the activation of natural pneumococcal action of human serum (6, 7).

Tests with blood obtained after administration of glucose sulfapyridine by various routes and of sodium sulfapyridine intravenously

Glucose sulfapyridine was given intravenously, subcutaneously, and orally, and blood samples taken at arbitrary intervals thereafter were tested for their action on Type III pneumococci. In addition, a number of samples of blood obtained from the glucose-sulfapyridine recipients were allowed to stand at 37° C for varying lengths of time and the tests repeated. Tests were also made with the blood of 2 subjects after the intravenous injection of sodium sulfapyridine. The results of all these tests are shown in Table II.

After parenteral injection of the glucose-sulfa-

TABLE II

Pneumococcal tests with defibrinated human blood after administration of glucose sulfapyridine solution or of sodium sulfapyridine

Subject	Drug	Route	Amount	Hours after administration	Hours at 37° C. before test	Sulfapyridine concentration		Growth inhibition		Pneumococcal action after 48 hours	
						Free	Total	24 hours	48 hours	Inoculum	Growth
S	G.S.P.	IV	5.0	0.5	0	11.9	15.2	10 ⁸	0		
				0.5	3.5			10 ⁸	10		
				0.5	14			10 ⁸	10		
				0.5	0	6.0	6.9	10 ⁸	10		
				0.5	0	2.6	3.4	0	0		
S	G.S.P.	IV	5.0	0.5	0	16.7	16.7	10	0		
				0.5	0	10.0	10.0	10	0		
				0.5	0	10.0	10.0	10	0		
				0.5	0	10.0	10.0	10	0		
				0.5	0	10.0	10.0	10	0		
A	G.S.P.	IV	4.75	1.0	0	14.0	15.4	10	10		
				1.0	26	2.0	2.3	10 ⁸	10		
				1.0	0	2.0	2.3	10	0		
				1.0	26	2.0	2.3	10	0		
				1.0	26	2.0	2.3	10	0		
Fo	G.S.P.	IV	4.75	1.0	0	13.4	16.0	0	0		
				3.0	0	4.6	4.7	0	0		
Fo	G.S.P.	S.C.	4.75	3.0	0	5.1	6.3	0	0		
				3.0	26	5.1	6.3	0	0		
K	G.S.P.	S.C.	9.5	3.0	0	10.2	12.5	10	10		
				3.0	0	10.2	12.5	10	10		
D	G.S.P.	P.O.	4.75	3.0	0	T	1.9	10 ⁸	10		
				3.0	26	T	1.9	10	0		
M	G.S.P.	P.O.	17.5†	48	0	19.7	22.2	10 ⁸	10 ⁸	10 ⁸	0
				48	0	15.4	18.7	10 ⁸	10 ⁸	10 ⁸	0
				96	0	6.9	9.6	10 ⁸	10 ⁸	10 ⁸	0
				96	0	6.9	9.6	10 ⁸	10 ⁸	10 ⁸	0
				96	0	6.9	9.6	10 ⁸	10 ⁸	10 ⁸	0
A	NaSP	IV	4.75	1.0	0	9.9	9.9	10 ⁸	10 ⁸	10 ⁸	12
				3.0	0	8.8	9.1	10 ⁸	10 ⁸	10 ⁸	3
Fo	NaSP	IV	4.75	1.0	0	9.9	11.1	10 ⁸	10 ⁸	10 ⁸	150
				3.0	0	7.6	8.9	10 ⁸	10 ⁸	10 ⁸	17

* Second injection of 5 grams 5 hours after the first

† From beginning of administration—each dose given in 1 hour except in Subject S who received it in 1½ hours. All parenteral injections were given in 500 cc. of normal saline

‡ 5 grams then 2.5 grams 4 hourly for 5 doses. Hours given are after the last dose. Fluids were given liberally during this period.

pyridine solution practically no pneumococcal action was noted even when high concentrations of drug were present in the blood. After preliminary incubation of these blood samples at 37° C., varying degrees of growth inhibition occurred depending on the level of the drug and the length of time allowed for incubation. In subject A the freshly shed blood on two occasions killed 10 diplococci and no killing occurred following incubation but the growth of a large number was inhibited. This again suggests that as with the *in vitro* experiment the small amount of killing was due to the immune mechanism rather than to the

action of the drug, the activity being abolished by the inactivation of complement and perhaps the death of leukocytes in the process of incubation

Following the intravenous injection of sodium sulfapyridine there was marked bacteriostatic and moderate bactericidal action comparable to the effects noted from the addition of this drug *in vitro* as noted above. This was also similar to the action of sulfapyridine added to blood *in vitro* (1) or when present in blood after its oral administration (2)

The results of the tests carried out with the blood taken after the oral administration of the glucose-sulfapyridine solution were in sharp contrast to those already noted with the blood obtained after parenteral administration of the same solution. The pneumococcal action was the same as after the intravenous injection of the sodium salt or after the oral administration of the unaltered sulfapyridine. The high concentration maintained in the blood of M for such a long interval is associated with the delayed absorption which was shown to be characteristic of the glucose sulfapyridine when given orally (4)

DISCUSSION

The results of the bactericidal tests reported here and the studies on absorption and excretion (4) are in essential agreement. They indicate that a new compound is formed by boiling sulfapyridine in 50 per cent glucose. This glucose-sulfapyridine solution differs from the original sulfapyridine and also from the sodium sulfapyridine in the rate in which it is cleared by the kidneys after parenteral administration as well as in its distribution in the body fluids when given by such routes. The bactericidal tests show further that this glucose-sulfapyridine solution is inert when added *in vitro* or when given parenterally. On standing in blood at 37° C., some hydrolysis takes place releasing sufficient sulfapyridine to bring about varying degrees of growth inhibition but not enough to effect any appreciable killing of the Type III pneumococci used. The method used here for determining the concentration of free sulfapyridine also causes hydrolysis of the glucose compound giving values which include both free sulfapyridine and sulfapyridine combined with glucose.

RESULTS

Effect of glucose-sulfapyridine and sodium sulfapyridine when added to defibrinated blood in vitro

The results of pneumococcal tests with the blood of 2 subjects are given in Table I. The blood of subject S was used on two occasions, 1 week apart. On both occasions the addition of glucose-sulfapyridine solution in amounts equivalent to 10 mgm per 100 cc. (1:10,000) did not

TABLE I

Pneumococcal action of sodium sulfapyridine and of glucose-sulfapyridine when added to defibrinated human blood in vitro

Subject	Material added	Concentration	Growth inhibition		Pneumococcal action at 48 hours	
			24 hours	48 hours	Inoculum	Growth
S	0	10 mgm per 100 cc	0	0		
	G SP		10	0		
S*	0	10 10 50 } Glucose	0	0		
	G SP		0	0		
	SP		10 ⁵	10 ⁵	10 ⁵	96
	Glucose				10 ⁴	0
F	0	10 10 10 SP	10	10	10	10
	G SP		0	0		
	NaSP		10 ⁴	10 ⁴	10 ³	0
	SP		10 ⁵	10 ⁵	10 ⁵ 10 ⁴	1200 0
F†	0	10 10	0	0		
	G SP		10 ²	0		
	NaSP		10 ³	10 ⁴	10 ⁴ 10 ² 10 ³	8 8 0

* Blood taken 1 week later

† Same blood kept at 37° C for 30 hours. The drugs were added prior to incubation

Explanation of Tables I and II

Amount In the case of the glucose-sulfapyridine this represents total amounts of sulfapyridine in the solution

Concentration Given in mgm per 100 cc. When added *in vitro*, the final concentration is given, except the glucose, for which the added concentration is given

Growth inhibition Numbers represent the largest inoculum which showed no color change

Pneumococci killed Listed only when inhibition was noted. Growth = number of colonies grown in agar pour plates. Inoculum refers to original number. Larger inocula yielded too many colonies to count, smaller ones yielded no growth

Abbreviations SP = sulfapyridine

G SP = glucose-sulfapyridine solution

NaSP = sodium sulfapyridine

I V = intravenous

SC = subcutaneous

P O = oral

result in any pneumococcal activity. That this lack of activity was not due to the presence of glucose as such was shown by the fact that, when both glucose and sulfapyridine were added in the same proportions as those originally present in the glucose-sulfapyridine solution, there was marked pneumococcal action. The extent of this action was the same as when no glucose was added (1).

The blood of subject F killed 10 diplococci when first tested. The same fresh blood showed no pneumococcal activity when glucose sulfapyridine was added, but considerable numbers were killed and the growth of even larger numbers was inhibited after the addition of sulfapyridine or its sodium salt.

Effect of incubation at 37° C

Duplicate control tests and tests with the addition of glucose sulfapyridine and sodium sulfapyridine were carried out with the blood of subject F after incubation for 30 hours before the organisms were added. There was no bactericidal activity in the blood without the drug. Slight inhibition of growth for 24 hours occurred in the blood containing glucose-sulfapyridine, while inhibition and killing in the blood containing sodium sulfapyridine were essentially the same as in the fresh blood.

The loss of the small amount of pneumococcus killing in the blood of subject F after incubation is consistent with the destruction of complement which is essential for the activation of natural pneumococcal action of human serum (6, 7).

Tests with blood obtained after administration of glucose sulfapyridine by various routes and of sodium sulfapyridine intravenously

Glucose sulfapyridine was given intravenously, subcutaneously, and orally, and blood samples taken at arbitrary intervals thereafter were tested for their action on Type III pneumococci. In addition, a number of samples of blood obtained from the glucose-sulfapyridine recipients were allowed to stand at 37° C for varying lengths of time and the tests repeated. Tests were also made with the blood of 2 subjects after the intravenous injection of sodium sulfapyridine. The results of all these tests are shown in Table II.

After parenteral injection of the glucose-sulfa-

TABLE II

Pneumococcal tests with defibrinated human blood after administration of glucose sulfapyridine solution or of sodium sulfapyridine

Subject	Drug	Route	Amount	Hours after administration	Hours at 37° C. before test	Sulfapyridine concentration		Growth inhibition		Pneumococcal action after 48 hours	
						Free	Total	24 hours	48 hours	Inoculum	Growth
S	G.SP	IV	5.0	0.5	0	11.9	13.2	10 ⁴	0	10	8 8 8
				0.5	0	11.9	13.2	10 ⁴	0	10	
				0.5	14	6.0	6.9	10 ⁴	0	10	
				1.0	0	2.0	3.4	10 ⁴	0	10	
				0.5	0	16.7	16.7	10 ⁴	0	10	
S	G.SP	IV	5.0	0.5	0	10.0	10.0	10	0	10	0
A	G.SP	IV	4.75	1.0	0	14.0	15.4	10 ⁴	0	10	0
				1.0	26	2.0	2.3	10 ⁴	0	10	
				3.0	26	0	0	0	0	10	
Fo	G.SP	IV	4.75	1.0	0	13.4	16.0	0	0	10	0
				3.0	0	4.6	4.7	0	0		
Fo	G.SP	S.C.	4.75	3.0	0	3.1	6.3	0	0	10	0
K	G.SP	S.C.	9.5	3.0	0	10.3	12.5	10	10	10	0
D	G.SP	P.O.	4.75	3.0	0	T	1.9	10 ⁴	10	10	0
				3.0	26	0	0	0	0	10	
M	G.SP	P.O.	17.5†	2	0	19.7	22.2	10 ⁴	10 ⁴	10 ⁴	0
				13.5	0	15.4	18.7	10 ⁴	10 ⁴	10 ⁴	4
				9.5	0	6.9	9.6	10 ⁴	10 ⁴	10 ⁴	0
A	NaSP	IV	4.75	1.0	0	9.9	9.9	10 ⁴	10 ⁴	10 ⁴	11
				3.0	0	8.8	9.1	10 ⁴	10 ⁴	10 ⁴	
Fo	NaSP	IV	4.75	1.0	0	9.9	11.1	10 ⁴	10 ⁴	10 ⁴	150
				3.0	0	7.6	8.9	10 ⁴	10 ⁴	10 ⁴	
				3.0	0	7.6	8.9	10 ⁴	10 ⁴	10 ⁴	

* Second injection of 5 grams 5 hours after the first.

† From beginning of administration—each dose given in 1 hour except in Subject S who received it in 1½ hours. All parenteral injections were given in 500 cc. of normal saline.

‡ 3 grams, then 2.5 grams, 4 hourly for 5 doses. Hours given are after the last dose. Fluids were given liberally during this period.

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action of the drug the activity being abolished by the inactivation of complement and perhaps the death of leukocytes in the process of incubation.

Following the intravenous injection of sodium sulfapyridine, there was marked bacteriostatic and moderate bactericidal action comparable to the effects noted from the addition of this drug *in vitro* as noted above. This was also similar to the action of sulfapyridine added to blood *in vitro* (1) or when present in blood after its oral administration (2).

The results of the tests carried out with the blood taken after the oral administration of the glucose-sulfapyridine solution were in sharp contrast to those already noted with the blood obtained after parenteral administration of the same solution. The pneumococcal action was the same as after the intravenous injection of the sodium salt or after the oral administration of the unaltered sulfapyridine. The high concentration maintained in the blood of M for such a long interval is associated with the delayed absorption which was shown to be characteristic of the glucose sulfapyridine when given orally (4).

DISCUSSION

The results of the bactericidal tests reported here and the studies on absorption and excretion (4) are in essential agreement. They indicate that a new compound is formed by boiling sulfapyridine in 50 per cent glucose. This glucose-sulfapyridine solution differs from the original sulfapyridine and also from the sodium sulfapyridine in the rate in which it is cleared by the kidneys after parenteral administration, as well as in its distribution in the body fluids when given by such routes. The bactericidal tests show further that this glucose sulfapyridine solution is inert

When the glucose-sulfapyridine solution is given orally, on the other hand, the material that is absorbed into the blood has the same properties, as regards both its renal clearance and its bactericidal action as free sulfapyridine. Here again, the compound is presumably hydrolyzed after ingestion, freeing sulfapyridine in an active form which then has the same clearance and bactericidal properties as uncombined sulfapyridine or its sodium salt. This hydrolysis after oral ingestion presumably takes place slowly, since absorption of the sulfapyridine into the blood has been shown to be considerably delayed as compared with the absorption of sulfapyridine as such (4).

In this study as in the others (1, 2), simultaneous tests for opsonins were carried out and the pneumococcal action of sulfapyridine was shown to be entirely independent of phagocytosis. The data are omitted since the results were negative throughout.

SUMMARY AND CONCLUSIONS

The bactericidal action of a 10 per cent solution of sulfapyridine in 50 per cent glucose was tested against a susceptible strain of Type III pneumococcus in human blood *in vitro*, and after its administration by the intravenous, subcutaneous and oral routes.

When added *in vitro* and after its parenteral administration this solution was found to be essentially inert. Blood to which similar amounts of sodium sulfapyridine are added *in vitro* or blood which is obtained after the intravenous administration of this compound has bacteriostatic and pneumococcal properties similar to those of sulfapyridine.

After oral ingestion of the glucose-sulfapyridine solution the bactericidal power of the blood is the same as that resulting from equivalent concentrations of free sulfapyridine. Absorption, however, is slower than after sulfapyridine taken orally.

These studies were carried out with the technical assistance of Mildred W. Barnes and Claire Wilcox. The chemical determinations were made by Margaret A. Adams and Nancy E. Marean.

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THE DIODRAST CLEARANCE AND RENAL BLOOD FLOW IN TOXEMIAS OF PREGNANCY

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Renal ischemia, produced by artificial constriction of the renal arteries results in a permanent hypertension the severity of which roughly parallels the degree of ischemia. (For reviews see Goldblatt (1) and Blalock and Levy (2))

Smith, Goldring and Chasis (3) have shown that at low plasma concentrations diodrast has a renal extraction ratio of nearly 100 per cent and that therefore the plasma clearance of diodrast gives the plasma flow through the kidney. In patients having essential hypertension Smith, Goldring Chasis and Ranges (4) report that they always found renal ischemia as shown by the diodrast clearance.

Toxemia of pregnancy seems to bear some definite relation to essential hypertension about half of post toxic patients will sooner or later show persistently elevated blood pressure Corwin and Herrick (5) have concluded that 'subacute hypertensive toxemia of pregnancy is the response of the woman with latent or declared cardiovascular disease to the strain of pregnancy'. Dill and Erickson (6) have produced an eclampsia like syndrome in dogs and rabbits by constriction of the renal arteries in pregnant animals.

In view of the foregoing considerations it would seem to be of fundamental importance to determine the diodrast clearance (and effective renal blood flow) in toxemia of human pregnancy.

MATERIAL AND METHODS

Patients were selected in whom the differential diagnosis could be made, with some confidence, between pre-eclampsia, nephritis and chronic hypertension complicated by pregnancy. By pre-eclampsia (and eclampsia) we mean an acute toxemia occurring late in pregnancy. It is characterized by a rapid and excessive gain in weight, hypertension, proteinuria and edema usually appearing in the order mentioned (and with or without convulsions). All these signs disappear rapidly following delivery (but not always permanently). Renal function tests such as the urea clearance, give normal results.

Nearly all patients studied in this group were primi gravidae. Our chronic hypertensives were all patients who have been observed in more than one pregnancy and followed between pregnancies. In most of these the urea clearance was normal. Chronic Bright's disease had either been so diagnosed before pregnancy or early in pregnancy. The criteria were hypertension, proteinuria, edema, hematuria diminished renal function as shown by urea clearance specific gravity and phenolsulphonphthalein excretion. Hypoproteinemia and hypercholesterolemia were also present in our cases.

Twelve normal pregnant and 9 normal non pregnant subjects were used as a control series. The first 17 of these have been reported elsewhere (Chesley and Chesley (7)).

Because of the possibility of stasis of urine in dilated ureters many of the pregnant and immediately postpartum patients were cystoscoped and ureteral catheters were inserted. A special catheter with 5 or 6 openings was placed in the bladder. From here on the procedure described by Smith Goldring and Chasis (3) was followed. A continuous infusion of 10 per cent dextrose containing 1 ml. of 35 per cent diodrast per 100 ml. was given at the rate of 10 ml. per minute for 10 minutes. The rate of infusion was then cut down to 4 ml. per minute. About half an hour after the beginning of the infusion the bladder was washed out with saline, and the urine collection period begun. At this time the first blood specimen was taken (oxalated). Three urine collections were made each over a period of about 20 minutes. The bladder was washed out twice each time to catch any urine which might have drained down the ureters. The washings were combined with the urine for analysis. At the end of the test the ureteral catheters were slowly pulled out with suction to get any urine that was possibly stagnating in the ureters. The second blood sample was taken midway in the test, and the third was obtained at the end. The following day (usually) the procedure was repeated without ureteral catheterization. These bladder urine collections were made over a period of about 45 minutes and the preliminary period of infusion was prolonged. (See Discussion below).

Diodrast was analyzed as iodine using a modification of Kendall's method as adapted by Smith, Goldring and Chasis (3). The use of glass beads was omitted, and the samples of plasma and diluted urine were dried overnight at 85 to 95 C. After fusion, the cooled melt was dissolved by adding water to the crucible and boiled carefully on a hot plate. The solution was

rectly into the analysis flask, and the crucible then rinsed six times with cold water brought to boil in the crucible. The final titration was made with 0.001 N sodium thio-sulphate. All reagents were freed of iodine by the methods described by McCullagh (8). Blank analyses were always negative. Frequent analyses of known solutions of both hippuran and potassium bionate in urine and plasma gave results close to theoretical. All determinations were done in duplicate.

The plasma clearance of endogenous creatinine was simultaneously determined by the methods of Folin (9). When the urine volume was in excess of 2 ml per minute, the maximal urea clearance was measured by the manometric procedures of Van Slyke (9).

toxic patients were *antepartum* and at the height of their toxemia.

In Table I are shown the plasma clearances of diodrast, endogenous "creatinine" and urea, the hematocrits, and the calculated renal blood flows, "filtration fractions" and urea extraction ratios. The "filtration fraction" was calculated by dividing the clearance of apparent creatinine by the diodrast clearance (renal plasma flow). We do not regard the endogenous creatinine clearance as a measure of glomerular filtration, though we had thought that it might give an approximation to

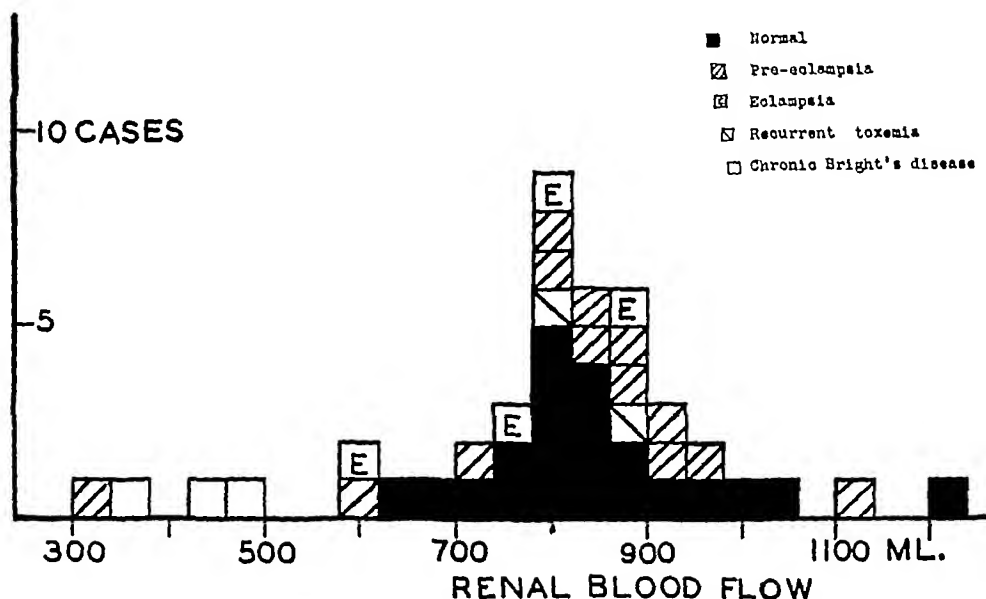


FIG 1 FREQUENCY DISTRIBUTIONS OF RENAL BLOOD FLOWS IN NORMAL SUBJECTS AND IN PRE-ECLAMPSIA, ECLAMPSIA, RECURRENT TOXEMIA, AND CHRONIC BRIGHT'S DISEASE COMPLICATING PREGNANCY

All observations on eclamptics were made after delivery, on the first, the third, and two on the eighth postpartum days

For the determination of the plasma fraction, 1 ml of well mixed blood was placed in a vaccine tube (designed by E M MacKay (9)) and centrifuged at 2500 r.p.m. for an hour. The volumes of cells and plasma were then read directly.

The effective renal blood flow was calculated by dividing the plasma clearance of diodrast by the plasma fraction. Hereafter, this figure will be called simply "renal blood flow". All clearances are corrected to the ideal body surface area of 1.73 sq m.

RESULTS

The renal blood flows for all subjects are shown in Figure 1. Unless specifically noted, all

the filtration rate. It would be hard to say what it does represent, since it seems to bear no constant relation to the urea clearance as it should if it were related simply to the glomerular filtration. Evidently all of the apparent creatinine of the plasma either is not filtered, or does not escape tubular reabsorption. Furthermore, the proportion so behaving varies from subject to subject. Our "filtration fraction" is therefore worthless.

The urea extraction ratio was computed by dividing the plasma clearance of urea (assumed to be nearly the same as the whole blood clearance) by the calculated renal blood flow. The extrac-

TABLE I

Toxic series of 24 patients

All data corrected to body surface area of 1.73 sq m. Uteral catheter specimens used in many cases. Blood pressure measurements made at time of test.

Degree of toxemia	Blood pressure	Plasma clearance of			Hematocrit	"Filtration fraction"	Urea extraction ratio	Renal blood flow
		Diodrast	Creatinine	Urea				
mm Hg	ml per minute	ml per minute	ml per minute	per cent	per cent	per cent	ml. per minute	
PRE ECLAMPTIC AND ECLAMPTIC								
Mild	180/110	709	66.0	99.0	38.5	9.4	8.7	1141
Mild	146/100	1022	66.0	106.2	40.2	8.4	6.2	1712
Mild	88/58	502	81.2	64.1	36.0	16.2	8.2	787
Mild	128/90	526	83.5		42.5	16.2		906
Mild	136/90	531	93.9	44.7	36.0	17.6	5.4	832
Mild	136/94	491	105.3		30.3	21.4		710
Mild	142/92	598	62.3	45.8	35.7	10.4	5.0	931
Mild	100/66	560	87.6		36.2	15.6		881
Mild	131/88	580	66.2	78.6	33.3	11.8	9.4	840
Mild	140/90	636	79.4	61.4	33.5	12.4	6.4	963
Mild antepartum	105/60	233	65.4	50.8	33.0	28.2	14.6	347
Mild postpartum	90/58	356	62.0	48.8	30.8	17.5	9.5	515
Eclampsia	120/90	580	86.1	71.3	28.5	14.9	9.3	770
Severe	170/100	540	53.0		33.2	9.9		810
Severe	170/100	637	67.7	65.6	34.7	12.6	6.7	985
Severe	150/90	411	51.7	40.7	32.7	12.6	6.7	611
Severe	138/92	601	87.2	68.5	33.0	14.4	7.7	895
Eclampsia antepartum	166/96	386	78.8	59.3	32.7	20.7	10.4	575
Eclampsia postpartum	116/80	597	67.0	77.4	31.7	11.2	8.8	875
Eclampsia postpartum	156/90	619	83.5	83.9	21.7	13.5	10.6	793
Mild with ptyelitis	125/90	542	139.3	64.4	25.3	37.8	14.1	459
Average		554	75.7	66.8	33.7	14.8	8.4	844
RECURRENT TOXEMIA								
	200/130	564	103.8	160.0	35.7	18.4	18.2	880
	138/80	583	57.5	56.3	28.0	9.9	6.9	809
CHRONIC BRIGHT'S DISEASE								
Moderate antepartum	200/130	514	58.2		36.2	18.9		488
Moderate postpartum	170/110	265	59.0	25.5	29.4	22.3	6.8	377
Moderate antepartum	150/100	324	47.9	42.0	27.0	13.8	9.4	448
Moderate postpartum	160/100	392	86.5		30.2	22.1		559
Moderate antepartum	150/96	278	46.3	31.4	26.0	16.7	8.4	375
Moderate postpartum	164/98	346	67.9	34.2	31.3	19.6	6.8	503

* Not averaged because of ptyelitis.

tion ratio thus obtained averages 8.7 per cent for all subjects. This agrees with the average of 8.7 per cent calculated from the data of Landis, Elsom, Bott and Shuels (10) and accords well with the data obtained by simultaneous analyses of systemic arterial and of renal venous bloods in dogs and rabbits (for review see Chesley and Chesley (7)).

The renal blood flow in patients with pre-eclampsia and eclampsia covers a wide range but is usually normal. In 1 case of mild pre-eclampsia the extraordinarily high blood flow of 1712 ml per minute was found. In another mild pre-eclampsia the flow was 347 ml per minute when the test was repeated 11 days later and after delivery the blood flow was 515 ml per minute. Clinically this patient had a typically pre-eclampsia toxemia, presaged in the eighth month by a rapid gain in weight followed by the appearance of hypertension and then of proteinuria and other signs and symptoms of pre-eclampsia. In one of the 4 eclampsia patients the diodrast clearance was done between convulsions, her renal blood flow at that time was 575 ml per minute. When checked 8 days after delivery the blood flow was 870 ml per minute. Perhaps the somewhat diminished level found at the time of the convulsions can be attributed to renal vascular spasm or possibly to the sedative (allonal). One patient is not included in the averages though the data are given in Table I. This patient was found to have a "ptyelitis" which quite possibly could affect the clearances since "ptyelitis" usually involves at least some of the renal parenchyma.

For the whole group of eclampsia and pre-eclampsia patients, the mean renal blood flow is very close to that of the normal group at 844 ml per minute with a standard deviation of 253 ± 27 ml. The standard error of the mean is 56.6 ml. All of the clearances studied were nearly the same as in the normal control group which averaged 850 ml, with a standard deviation of 126 ± 13 ml. The standard error of the mean for the normal group of 21 subjects was 27.6 ml. The "filtration fraction" and urea extraction ratio averaged very slightly higher for the toxemia patients than for the normals.

Two multiparous patients were studied in whom repeated toxemia of pregnancy had occurred and in whom blood pressures were always found to be elevated between pregnancies. Both were again toxemic and near term. The renal blood flows were normal—880 and 809 ml per minute (Table I).

Four patients with chronic Bright's disease were observed. At least 2 of these have nephrosclerosis. The results shown in Table II indicate

a diminished renal blood flow, averaging about half that of the control or pre-eclamptic subjects. The "filtration fraction" is increased, as observed by Smith, Goldring, Chasis and Ranges (4) in their cases of essential hypertension. Curiously enough, the urea extraction ratio is slightly lower than the average, or in other words, the urea clearance runs nearly parallel with the diodrast clearance. This disparate behavior of the clearances of endogenous "creatinine" and urea (in only 3 cases) suggests that perhaps there is an increased tubular permeability allowing back-diffusion of urea to the blood. An impairment in tubular function (secretory) has been demonstrated in essential hypertension by Smith *et al* (4). Landis, Elsom, Bott and Shiels (10) found that the urea clearance paralleled fairly well the hippuran clearance (as did the creatinine clearance).

TABLE II

Comparison of diodrast clearances with urine specimens obtained by ureteral catheters and by bladder catheter

Patient	1	2	3	4	5	6	7	8	9	Average
	ml	ml	ml	ml	ml*	ml	ml	ml	ml	ml
Ureteral catheter specimens	505	598	610	416	374	588	756	632	492	552
Bladder catheter specimens	543	702	518	393	568	532	588	555	607	556

* Patient received 2 capsules of nembutal at time of catheterization

To summarize, the renal blood flow is not affected (usually) in pre-eclampsia and eclampsia. In such toxemias, two diminished and one markedly increased renal flow have been observed in 20 cases. In chronic Bright's disease complicated by pregnancy, there is a marked diminution in the renal blood flow. This, in our cases, was accompanied by a decrease in the urea clearance.

There was no significant effect upon the blood pressure exerted by the small amounts of diodrast given. The blood pressure increased slightly in about as many cases as it fell. In only 1 case—the eclamptic whose diodrast clearance was done between convulsions—was the pressure change at all marked. In this case the blood pressure at the beginning of the test was 146/90, and at the end it was 100/70. (Wide fluctuations in blood pressure are characteristic of eclampsia and pre-eclampsia.)

DISCUSSION

Urine collections The quantitative collection of urine in the pregnant and puerperal woman is difficult because of bladder atony and ureteral dilatation, with consequent stagnation of urine. Furthermore, the bladder is often displaced downward and pockets are formed which are hard to drain by catheter. In the present study an effort has been made to make complete collections of bladder urine by using a multi-opening catheter. At each collection period the bladder was washed out twice with 20 ml of saline as recommended by Smith. External suprapubic pressure often aided in getting back the instilled saline.

Ureteral dilatation, found in about 85 per cent of pregnant women (Stander (11)), may occasionally be of such marked degree as to hold back from the bladder an hour's output of urine. Bladder urine collections cannot give reliable clearance calculations unless the blood level of the substance under study is essentially constant, and unless the degree of diuresis is nearly constant or high. Even then an occasional strong peristaltic wave in the ureter might sweep accumulated urine into the bladder.

In the tests in which bladder urines were used, an effort was made to minimize the vitiating factors mentioned. In all cases the blood level of diodrast was kept very nearly constant. (The greatest variation in any case, excepting the few in whom low clearances were encountered, and one variation of 27 per cent, was 13 per cent, the average maximal variation from lowest to highest observed blood levels was 9.2 per cent. Almost invariably the blood level of diodrast progressively but slowly increased.) The period of infusion before the beginning of the urine collection period was at least 40 minutes. Thus diodrast-containing urine would not be diluted by stagnating diodrast-free urine in the ureters. Moderate to high degrees of diuresis were maintained, sodium sulphate was not used. Urine collection periods lasted 45 minutes, three urines were collected.

As stated in the description of methods, ureteral catheterization was done in many of the pregnant patients. Since the catheters are small, some urine might pass down the ureters outside of the catheters. Such urine has been collected as described above. We feel that in these catheterized

patients the urine collections have been quantitative. However, another possible complication arises. Ureteral reflexes set up by the catheterization have been known to cause anuria, probably by renal circulatory disturbance. (No evidence of such disturbance has been observed in our present series) If so marked an effect as anuria can result from ureteral catheterization it might be that some lesser renal circulatory disturbance would often occur

As a double check first upon the reliability of bladder urine collections and second upon the possibility of ureteral reflex inhibition of renal blood flow the diodrast clearance was done twice on each of many of the patients. Usually the first clearance was done with ureteral catheter collections the following day (usually), or within a week, the clearance was repeated with bladder collections. As the data in Table II show the clearances may be done either way. In half the cases the bladder urines gave the higher clearances and in half the ureteral urine gave higher results. The total averages for the two groups are similar. The advantage of the ureteral catheterization lies in the satisfying agreement between successive clearances. The discomfort to a pregnant patient near term however outweighs this advantage.

Hypertensive complications of pregnancy Despite the considerations outlined in the introduction, which seemed to promise so well for renal ischemia as a cause (or even the principal cause) of toxemia of pregnancy, the renal blood flow seems to be characteristically normal in pre-eclampsia, eclampsia and 'recurrent toxemia'. Moreover this finding pushes back another step the defenders of the old hypothesis that renal insufficiency underlies toxemia of pregnancy. After a century of slow stubborn retreat from the position that eclampsia was uremic in nature the argument of this school must now be based upon how much the renal blood flow could be if all the blood vessels of the kidney were maximally dilated.

Many of the patients whom we have diagnosed as 'mild pre-eclampsia' would be called 'low reserve kidney' by obstetricians using that classification. If one had previously doubted that a renal deficiency underlay the mild toxemia called

'low reserve kidney' his doubts must now be increased by the demonstration of a normal renal blood flow in such cases.

Since the renal blood flow characteristically seems to be normal in pre-eclampsia and eclampsia, one might expect renal function tests to give normal results when extra renal factors are taken into account. Such is the case (Chesley (12, 13)).

SUMMARY AND CONCLUSIONS

In eclampsia, pre-eclampsia and recurrent toxemia of pregnancy, the renal blood flow as measured by the diodrast clearance, seems to be characteristically normal. There is a wider range above and below average than was found in the normal subjects.

In the few cases of chronic nephritis complicating pregnancy studied the calculated renal blood flow is considerably below the levels found in normal subjects.

The urea extraction ratio averages 87 per cent as calculated from the plasma clearance of urea divided by the renal blood flow.

We wish to acknowledge our indebtedness to Drs. S. A. Cosgrove, J. F. Norton, and E. G. Waters for their permission to use patients from their services, and for reading the typescript. Dr. P. O. Hall has allowed us to study several of his private patients. Frances Orsato and Peter Marotta did many of the blood and urine urea analyses.

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OBSERVATIONS ON THE ETIOLOGIC RELATIONSHIP OF ACHYLIA GASTRICA TO
PERNICIOUS ANEMIA. VIII FURTHER STUDIES OF THE PROTEOLYTIC
ACTIVITY OF NORMAL HUMAN GASTRIC JUICE *IN VITRO*, AND
THE LIMITATIONS OF THE METHOD IN
PERNICIOUS ANEMIA¹

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It has been shown in previous studies (1, 2) that normal human gastric juice contains a proteolytic enzyme capable of hydrolyzing casein to the proteose stage in an alkaline medium, but not at hydron concentrations below pH 4. This range of activity and certain other properties would seem to distinguish the enzyme from pepsin, while the failure of the enzyme to produce large amounts of amino-nitrogen within 24 hours appears to rule out trypsin and erepsin, acting in their generally accepted manner.

Although no assertion can be made that this proteolytic activity is identical with that of the so-called intrinsic factor as detected clinically, it is of interest that the proteolytic activity in question is retained or destroyed under certain circumstances which affect the clinical activity of the intrinsic factor in a similar fashion (2). The present communication presents further resemblances of this nature. It also includes data indicating the limitations of the method of the *in vitro* hydrolysis of casein as a means of determining the activity in question in samples of the gastric contents of patients with pernicious anemia.

A. Effect of adsorption with Lloyd's reagent and of dialysis upon normal human gastric juice

Gastric juice visibly free from bile was collected from normal persons after the administration of 0.5 mgm of histamine phosphate. This fresh gastric juice or subsequent modifications were brought to approximately pH 7.4 with normal sodium hydroxide. Fifty ml of such ma-

terials were rapidly mixed in an Erlenmeyer flask with 50 ml. of a 1 per cent neutral casein solution prepared as previously described (2). The mixture was adjusted to exactly pH 7.4 and 2 ml of toluol were added. The flasks were then set in a constant temperature bath at 37.5° C for 24 hours. Samples of the digestion mixture were removed immediately and after 24 hours and analyzed for the total amount of nitrogen not precipitable by 10 per cent trichloroacetic acid and for the amino-nitrogen content respectively. From these determinations the amount of total filtrable nitrogen and of amino-nitrogen produced in 24 hours was calculated.

1. *Adsorption of normal human gastric juice with Lloyd's reagent.* Helmer and Fouts (3) have shown that after normal human gastric juice is shaken with from 10 to 15 grams of Lloyd's reagent its hematopoietic power in pernicious anemia when fed daily with 4.5 grams of powdered Liver Extract Lilly (N. N. R.) as a source of extrinsic factor is reduced by one-half to two thirds. In our (2) hands however after this procedure the gastric juice retained *in vitro* no detectable proteolytic activity. It was suggested that the residual activity of the gastric juice found by Helmer and Fouts might have been due to a greater content of mucus which might have interfered with the adsorptive power of the Lloyd's reagent. Since the adsorption by Lloyd's reagent seemed to offer a reasonable method of concentration of the enzyme it was decided to repeat both the *in vitro* and the clinical observations.

Accordingly, samples of 100 ml of unneutralized normal human gastric juice were filtered through gauze, then shaken once with 10 grams of Lloyd's reagent and filtered or centrifuged free of the adsorbent. In certain instances the acid

¹ The expenses of this investigation were defrayed in part by the J. K. Lilly Gift to the Harvard Medical School.

² Graduate Fellow of the Belgian American Educational Foundation, 1937-1939.

gastric juice was neutralized before adsorption or was treated twice with 15 grams of Lloyd's reagent. In one experiment 65 grams of commercial mucin were added to 100 ml of gastric juice before adsorption with Lloyd's reagent. The variations as well as the results of the different experimental techniques are given in Table I. The data given there show that, while untreated gastric juice produced marked increases in total filtrable nitrogen, those samples adsorbed either once or twice with Lloyd's reagent were found to be inert in this respect, regardless of the reaction of the gastric juice at the time the adsorption was carried out. The results of experiments 93a and 93b indicate that the addition of mucin to the gastric juice permitted retention of about 25 per cent of the original proteolytic power when extracted only once with Lloyd's reagent, as Helmer and Fouts had done.

A clinical test of gastric juice which had been

TABLE I

Proteolytic activity of normal human gastric juice on casein at pH 7.4 after adsorption with Lloyd's reagent

Experiment number	Conditions during adsorption of 100 ml of gastric juice with Lloyd's reagent			Subsequent proteolytic activity	
	Lloyd's reagent	Number of adsorptions	pH during adsorption	Increase in nitrogen in trichloroacetic acid filtrates	Increase in amino-nitrogen by formal titration
	grams			mgm per 100 ml digest in 24 hours	
65a	0	0		53.92	1.82
65b	10	1	7.4	0.34	0.00
67a	0	0	0	15.50	0.00
67b	10	1	1.8	0.00	0.00
68a	0	0		47.20	0.30
68b	10	1	7.4	0.24	0.50
70a	0	0		12.29	0.70
70b	10	1	7.4	1.14	0.00
70c	10	1	1.2	0.00	0.00
93a*	0	0		23.00	1.38
93b*	10	2	1.2	5.20	0.00
133a	0	0		40.30	1.40
133b	10	2	1.8	0.48	0.00
275a	0	0		22.97	0.28
275b	15	2	1.8	0.00	0.00

* 6.5 grams of mucin per 100 ml of gastric juice were added in this experiment. Both control and the portion of the sample adsorbed with Lloyd's reagent were exposed to pH 10 for 2 hours before incubation at pH 7.4 for 24 hours.

TABLE II

Effect of daily administration to patients with pernicious anemia of 200 grams of beef muscle with 100 ml of normal human gastric juice after extraction with Lloyd's reagent and after dialysis, respectively

First periods Daily administration of preparations indicated below				
Days of treatment	Case 79		Case 80	
	100 ml of gastric juice after extraction twice with 15 grams of Lloyd's reagent		100 ml of gastric juice after dialysis for 3 days at 4°C	
	Red blood cells	Reticulocytes	Red blood cells	Reticulocytes
	millions per c mm	per cent	millions per c mm	per cent
0	1.59	1.2	1.50	1.2
2	1.53	1.0	1.39	3.0
4	1.59	1.2	1.40	3.5
6	1.55	2.2	1.59	5.8
8	1.41	4.0	1.84	15.8
10	1.46	5.6	1.94	6.4
12	1.27	5.0	2.00	5.8

Second periods Daily administration of preparations indicated below

	Same as above except without extraction with Lloyd's reagent	
	millions per c mm	per cent
2	1.28	5.0
4	1.47	15.6
6	1.86	30.6
8	2.18	30.2
10	2.57	15.4

extracted twice with 15 grams of Lloyd's reagent was then carried out. In accordance with a technique previously demonstrated (4) to be effective in the detection of intrinsic factor, a patient with pernicious anemia (Table II, Case 79) was fed 100 grams of finely divided beef muscle and 50 ml of neutralized gastric juice, after extraction with Lloyd's reagent, at both the noon and evening meals each day for a period of 12 days. During this time there was no clinical improvement or laboratory evidence of significantly increased blood formation. During a second period of 10 days the conditions were identical except that freshly neutralized gastric juice was substituted for gastric juice which had been extracted with Lloyd's reagent. The patient then responded clinically and a reticulocyte peak of 30.6 per cent was attained on the sixth day with an

increase within 10 days of over a million cells above the initial count of 1,270 000 red blood cells per cu.mm. From this it appears that *in vitro* evidence of proteolysis as well as clinical evidence of intrinsic factor activity, was absent after treatment of normal human gastric juice with Lloyd's reagent in the manner described.

2 Dialysis of normal human gastric juice It has been shown by Helmer and Fouts (3) and by Helmer, Fouts and Zervas (5) that the intrinsic factor of normal human gastric juice does not pass through an ultrafilter. Goldhamer and Kyer (6) have more recently shown that the precipitate formed by saturation of gastric juice with ammonium sulfate and thereafter dialyzed for 8 hours is potent as a source of intrinsic factor when fed with a suitable extrinsic factor to patients with pernicious anemia.

In order to avoid possible changes in the diffusibility of the intrinsic factor and the introduction of extrinsic nitrogen preliminary precipitation with ammonium sulfate was omitted and unprecipitated normal human gastric juice was dialyzed in cellophane sacks at a temperature below 6° C for 3 or 5 days respectively. As dialysis proceeded a white precipitate, presumably the euglobulin of the gastric juice, appeared. The contents of the sack were removed at the end of this period, adjusted if necessary to the original volume with distilled water, and thoroughly mixed. The material was then neutralized and a 50 ml sample was incubated with an equal volume of casein solution at pH 7.4 in the usual manner. The results are given in Table III experiment 178b. There it will be observed that the dialyzed gastric juice produced significant amounts of total filtrable nitrogen in a manner comparable to that of unmodified gastric juice. In experiment 179b the experiment was repeated except that the contents of the sack were filtered through paper before mixing with the casein solution. In experiment 180a the precipitate left on the paper was resuspended in a volume of distilled water equal to that of the sample before filtration and incubated with an equal quantity of casein solution. Both the filtrate and the precipitate possessed distinct proteolytic power.

In order to provide a clinical test for intrinsic factor, a patient with pernicious anemia (Table II, Case 80) was fed daily 100 ml of dialyzed

TABLE III
Proteolytic activity of normal human gastric juice on casein at pH 7.4 after dialysis

Experiment number	Preparation of gastric juice	Increase in nitrogen in trichloroacetic acid filtrates	Increase in amino-nitrogen by formal titration
		mgm. per 100 ml. digest in 4 hours	
178b	Contents of sack after dialysis at 4° C for 5 days	53.04	0.56
179b	Filtrate from contents of sack after dialysis at 4° C. for 3 days	57.63	0.56
180a	Precipitate from contents of sack after dialysis at 4° C for 3 days	51.51	0.42

gastric juice and 200 grams of beef muscle in the same manner as in the previous clinical observation. During a period of 12 days the patient responded clinically, and a reticulocyte peak of 15.8 per cent appeared on the eighth day. The red blood cells initially were 1,500 000 per cu.mm. and reached 2 000 000 on the twelfth day. These data entirely confirm the clinical observations of others (3, 5, 6) that intrinsic factor does not pass through a semipermeable membrane and exclude the possible complication in interpretation introduced through the use of ammonium sulfate before dialysis by Goldhamer and Kyer (6).

DISCUSSION

The parallelism between the clinical activity of the so-called intrinsic factor and the proteolytic activity of normal human gastric juice under discussion has been further extended to include a similar action of Lloyd's reagent and of dialysis. Nevertheless, this does not constitute proof of identity or imply as has been proved to the contrary by Wintrobe (7) that casein is a suitable extrinsic factor from a clinical point of view.

B The *in vitro* proteolytic activity of the gastric secretion in pernicious anemia

Until recently no observations had been made in this laboratory on the *in vitro* proteolytic activity of gastric juice other than from normal human subjects with the exception of the two instances previously reported (2) in which gastric juice was obtained from patients with pernicious

anemia In those observations, marked increases in amino-nitrogen were obtained in 24 hours with such bile-stained gastric contents It was then found that, after exposure of the material to pH 10 for 2 hours in order to diminish tryptic activity, both the total filtrable nitrogen and the amino-nitrogen production were greatly decreased These preliminary observations, together with the experiments reported by Lasch (8), suggested the desirability of further *in vitro* studies of the gastric secretion of patients with pernicious anemia The present investigations are thus an attempt to test the validity of the casein hydrolysis method as a means of assaying the activity in question in samples of the gastric contents in pernicious anemia A secondary consideration would obviously be whether such a method could serve as a diagnostic test for pernicious anemia

Accordingly, samples of the fasting achlorhydric gastric contents of each of 12 patients with pernicious anemia were obtained after the subcutaneous injection of 0.5 mgm of histamine phosphate In order to secure the necessary 45 ml of material, continuous suction for from 1 to 6 hours was needed In different patients, as shown in Table IV, the average rate of aspiration varied from 8 to 78 ml per hour In all but two instances bile was visibly present in the samples Thus regurgitation from the intestine must have both contaminated and augmented the apparent gastric secretion

Portions of the samples obtained were incubated in the usual manner with an equal volume of casein solution at pH 7.4 In only six instances, including the two in which no bile staining was visible, was the amino-nitrogen production less than 2 mgm in 24 hours, even after previous exposure of the gastric contents to alkali (Table IV, lower half) The gastric contents of the other 6 patients, even after exposure to alkali, produced amounts of amino-nitrogen greater than were developed by any of the samples of pure gastric juice from normal individuals previously reported (Table IV, upper half) Experiments 253a and 277a are typical of the very considerable production of amino-acid when exposure to alkali was not practiced These particular results resemble those previously obtained (2) with normal human gastric juice which had been purposefully contaminated with duodenal contents In no in-

TABLE IV

Proteolytic activity on casein at pH 7.4 of gastric contents of patients with pernicious anemia

Experiment number	Character of gastric contents		Preparation of gastric juice	Subsequent proteolytic activity	
	Amount obtained	Bile contamination		Increase in nitrogen in trichloroacetic acid filtrates	Increase in amino-nitrogen by formal titration
	milli-liters per hour			mgm. per 100 ml digest in 24 hours	

A. SAMPLES WITH WHICH AMINO-NITROGEN PRODUCTION IN 24 HOURS EXCEEDED 2 MGm. PER 100 ML.

253a	10	+	None	25.5	25.2
253b			2 hours exposure to pH 10	17.5	2.4
92a		+	None	49.8	12.0
92b			2 hours exposure to pH 10	38.9	6.0
241	8	+	2 hours exposure to pH 10	50.1	4.5
410	20	+	2 hours exposure to pH 10	54.6	6.02
411	38	+	2 hours exposure to pH 10	75.2	8.4
412	37	+	2 hours exposure to pH 10	80.6	2.1

B. SAMPLES WITH WHICH AMINO-NITROGEN PRODUCTION IN 24 HOURS WAS LESS THAN 2 MGm. PER 100 ML.

277a	10	+	None	85.8	15.82
277b			2 hours exposure to pH 10	21.8	1.7
414	10	+	2 hours exposure to pH 10	28.7	1.4
407	25	+	2 hours exposure to pH 10	42.4	0.8
245	13	+	2 hours exposure to pH 10	50.1	1.83
408	45	0	2 hours exposure to pH 10	19.8	0.8
415a	78	0	None	61.6	10.03
415b			2 hours exposure to pH 10	63.8	1.4

stance, even after exposure to alkali, was the production of total filtrable nitrogen by samples of the gastric contents of pernicious anemia patients distinctly less than certain values previously reported for normal gastric juice

Since, according to Northrop (9), only 70 to 80 per cent of trypsin in solution is destroyed by exposure to alkali, the influence of any considerable amount of enzymes regurgitated from the duodenum would remain a seriously interfering factor, even after such a significant reduction by exposure to alkali This supposition was confirmed by exposure to alkali of solutions containing equal parts by weight of commercial trypsin³

³ Trypsin, Pfanstiehl 175, Pfanstiehl Chemical Company, Waukegan, Illinois

and erepsin⁴ in a total concentration of one part per thousand. It was found to be possible to reduce subsequent amino-nitrogen production on casein at pH 7.4 in 24 hours from 12.04 and 10.08 to 0.56 and 2.24 mgm. respectively, in two experiments. Yet even after these significant reductions in the formation of amino nitrogen, the productions of total filtrable nitrogen were, respectively, 22.57 and 79.08 mgm in 24 hours. It was therefore obvious that, when trypsin and erepsin are present in significant amounts in gastric contents they cannot be sufficiently inhibited by exposure to alkali to render the use of the casein hydrolysis method adequate for the studies of the enzyme under discussion.

DISCUSSION

In contrast to its apparently successful use in the study of the enzyme activity of normal human gastric juice the systematic application of the casein hydrolysis technique to the gastric juice of patients with pernicious anemia appears to be impracticable. In the first place, the very slow rate of actual gastric secretion in pernicious anemia permits an unusual degree of contamination with mucus saliva or intestinal contents. Therefore, on the basis of this dilution effect alone the secretion obtained can scarcely be considered to represent a sample of gastric secretion in any quantitative sense comparable to that obtained from a normal stomach. In the second place, it is only on rare occasions that adequate samples of gastric contents can be obtained from such patients free from regurgitated intestinal enzymes. Exposure to alkali, as shown by experiments with solutions of trypsin and erepsin and with the specimens of contaminated gastric secretions from patients with pernicious anemia failed in most instances to reduce the amino nitrogen production to less than 2 mgm within 24 hours. This technique cannot therefore be used to eliminate the production of total filtrable nitrogen by such interfering enzymes when present in any considerable concentration.

Because of this difficulty the characteristics of only the two samples of gastric secretion from pernicious anemia patients which were visibly

free from bile (experiments 408 and 415) may be justifiably compared with previous results on normal gastric juice. After exposure to alkali there was in both, minimal production of amino-nitrogen but considerable ability to produce total filtrable nitrogen. Exposure to alkali in experiment 415b did not affect the production of total filtrable nitrogen. Accordingly this sample resembles normal gastric secretion, and because the amount of secretion 78 ml. in an hour was unusually great for the average patient with pernicious anemia (see Table IV), it is possible that the gastric secretion of this particular patient contained a greater amount of the enzyme in question than is usually contained in the gastric secretion of the typical pernicious anemia patient. With regard to the samples as a group even in the six in which exposure to alkali reduced the formation of amino-nitrogen to less than 2 mgm in 24 hours the production of total filtrable nitrogen was not distinguishably less than for normal gastric juice (2).

Although we attempt to draw no conclusions from the data as a whole because of the obvious experimental difficulties, it may not be out of place to discuss certain possible clinical difficulties in interpretation. Even if it is assumed that the *in vitro* activity of normal human gastric juice under discussion is identical with that of the so-called intrinsic factor it is not necessary to suppose that the absence of such activity will always be characteristic of or can invariably serve as a diagnostic test for pernicious anemia. It has been demonstrated that certain amounts of extrinsic factor (200 grams of beef muscle) are usually unable to cause significant blood production when administered daily in pernicious anemia (10). When however presumably larger amounts (7-11) of extrinsic factor (yeast preparations) are given certain patients at least display hematopoietic responses probably because of residual amounts of intrinsic factor in the gastric secretion. Because of the relatively insignificant volume of true gastric secretion in pernicious anemia the development of the disease is perhaps not incompatible with a significant concentration of intrinsic factor in the gastric juice of certain patients. Thus Goldhamer (12) has

⁴Erepsin Duodenal Digestive Ferment Company Detroit, Michigan.

TABLE IV

Proteolytic activity on casein at pH 7.4 of gastric contents of patients with pernicious anemia

Experiment number	Character of gastric contents		Preparation of gastric juice	Subsequent proteolytic activity	
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	mls. liters per hour			mgm. per 100 ml. digest in 24 hours	

A. SAMPLES WITH WHICH AMINO-NITROGEN PRODUCTION IN 24 HOURS EXCEEDED 2 MG. PER 100 ML.

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415b			2 hours exposure to pH 10	63.3	1.4

In those observations, marked increases in amino-nitrogen were obtained in 24 hours with such bile stained gastric contents. It was then found that, after exposure of the material to pH 10 for 2 hours in order to diminish tryptic activity, both the total filtrable nitrogen and the amino-nitrogen production were greatly decreased. These preliminary observations, together with the experiments reported by Lasch (8), suggested the desirability of further *in vitro* studies of the gastric secretion of patients with pernicious anemia. The present investigations are thus an attempt to test the validity of the casein hydrolysis method as a means of assaying the activity in question in samples of the gastric contents in pernicious anemia. A secondary consideration would obviously be whether such a method could serve as a diagnostic test for pernicious anemia.

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stance, even after exposure to alkali, was the production of total filtrable nitrogen by samples of the gastric contents of pernicious anemia patients distinctly less than certain values previously reported for normal gastric juice.

Since, according to Northrop (9), only 70 to 80 per cent of trypsin in solution is destroyed by exposure to alkali, the influence of any considerable amount of enzymes regurgitated from the duodenum would remain a seriously interfering factor, even after such a significant reduction by exposure to alkali. This supposition was confirmed by exposure to alkali of solutions containing equal parts by weight of commercial trypsin³

³ Trypsin, Pfanstiehl 175, Pfanstiehl Chemical Company, Waukegan, Illinois

and erepsin⁴ in a total concentration of one part per thousand. It was found to be possible to reduce subsequent amino-nitrogen production on casein at pH 7.4 in 24 hours from 12.04 and 10.08 to 0.56 and 2.24 mgm., respectively, in two experiments. Yet even after these significant reductions in the formation of amino-nitrogen, the productions of total filtrable nitrogen were respectively 22.57 and 79.08 mgm. in 24 hours. It was therefore obvious that when trypsin and erepsin are present in significant amounts in gastric contents they cannot be sufficiently inhibited by exposure to alkali to render the use of the casein hydrolysis method adequate for the studies of the enzyme under discussion.

DISCUSSION

In contrast to its apparently successful use in the study of the enzyme activity of normal human gastric juice the systematic application of the casein hydrolysis technique to the gastric juice of patients with pernicious anemia appears to be impracticable. In the first place the very slow rate of actual gastric secretion in pernicious anemia permits an unusual degree of contamination with mucus saliva or intestinal contents. Therefore on the basis of this dilution effect alone the secretion obtained can scarcely be considered to represent a sample of gastric secretion in any quantitative sense comparable to that obtained from a normal stomach. In the second place, it is only on rare occasions that adequate samples of gastric contents can be obtained from such patients free from regurgitated intestinal enzymes. Exposure to alkali as shown by experiments with solutions of trypsin and erepsin and with the specimens of contaminated gastric secretions from patients with pernicious anemia failed in most instances to reduce the amino-nitrogen production to less than 2 mgm. within 24 hours. This technique cannot therefore be used to eliminate the production of total filtrable nitrogen by such interfering enzymes when present in any considerable concentration.

Because of this difficulty the characteristics of only the two samples of gastric secretion from pernicious anemia patients which were visibly

free from bile (experiments 408 and 415) may be justifiably compared with previous results on normal gastric juice. After exposure to alkali, there was in both, minimal production of amino-nitrogen but considerable ability to produce total filtrable nitrogen. Exposure to alkali in experiment 415b did not affect the production of total filtrable nitrogen. Accordingly, this sample resembles normal gastric secretion, and because the amount of secretion, 78 ml. in an hour, was unusually great for the average patient with pernicious anemia (see Table IV) it is possible that the gastric secretion of this particular patient contained a greater amount of the enzyme in question than is usually contained in the gastric secretion of the typical pernicious anemia patient. With regard to the samples as a group, even in the six in which exposure to alkali reduced the formation of amino-nitrogen to less than 2 mgm. in 24 hours the production of total filtrable nitrogen was not distinguishably less than for normal gastric juice (2).

Although we attempt to draw no conclusions from the data as a whole because of the obvious experimental difficulties it may not be out of place to discuss certain possible clinical difficulties in interpretation. Even if it is assumed that the *in vitro* activity of normal human gastric juice under discussion is identical with that of the so-called intrinsic factor it is not necessary to suppose that the absence of such activity will always be characteristic of or can invariably serve as a diagnostic test for pernicious anemia. It has been demonstrated that certain amounts of extrinsic factor (200 grams of beef muscle) are usually unable to cause significant blood production when administered daily in pernicious anemia (10). When however presumably larger amounts (7-11) of extrinsic factor (yeast preparations) are given, certain patients at least display hematopoietic responses probably because of residual amounts of intrinsic factor in the gastric secretion. Because of the relatively insignificant volume of true gastric secretion in pernicious anemia, the development of the disease is perhaps not incompatible with a significant concentration of intrinsic factor in the gastric juice of certain patients. Thus Goldhamer (12) has

⁴Erepsin, Duodenal Digestive Ferment Company Detroit Michigan

demonstrated that when 150 ml of gastric contents derived from pernicious anemia patients were given daily with 200 grams of beef muscle, moderate effects on blood production in another patient with pernicious anemia were observed. Accordingly, in explaining the development of any one case of macrocytic anemia responsive to treatment with liver extract, account must be taken not only of variations in the amount of intrinsic factor but also in the amount of extrinsic factor ingested (4, 13, 14), of difficulty with intestinal absorption (4, 15), and possibly of positive or toxic factors of intestinal origin (16, 17). For this reason, any test applied to gastric secretion alone cannot be expected to be of absolute diagnostic significance.

If, however, the activity of normal human gastric juice described by Lasch (8) and ourselves (1, 2) is in reality a measure of the concentration of the so-called intrinsic factor, a satisfactory method for its quantification would still be of interest. Jones and Wilkinson (18) state that with the method of Lasch they have been unable to detect differences between normal and pernicious anemia gastric juice. In any case, because intestinal contents are usually present in samples of the gastric juice of patients with pernicious anemia, a satisfactory method of dealing with such material must distinguish the specific enzyme activity under investigation from that of enzymes regurgitated from the intestine.

CONCLUSIONS

1 Like the so-called intrinsic factor, the agent responsible for the proteolytic activity *in vitro* of normal human gastric juice at pH 7.4 may be completely removed by adsorption with Lloyd's reagent. It is unable to penetrate a semipermeable membrane.

2 Because of the usual presence of interfering enzymes from the intestine, the *in vitro* method was unsatisfactory for determining in pernicious anemia the amount of proteolysis which could be ascribed to the proteolytic agent in normal human gastric juice referred to above.

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RENAL FUNCTION AND THE AZOTEMIA FOLLOWING HEMATEMESIS¹

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Elevation of the blood urea nitrogen following massive hemorrhage from the stomach and duodenum is a frequent occurrence (1) Its mechanism is not the same as that associated with high intestinal obstruction, as it occurs in the absence of any vomiting (2) (*eg* in the presence of melena alone⁴), and is associated with a normal or increased blood chloride concentration (3 4 5) and a normal carbon dioxide combining power of the blood (4 5) The factors contributing to this increase of the urea nitrogen content of the blood have been said to comprise shock dehydration, starvation, renal insufficiency and absorption of decomposition products of the blood liberated in the intestinal tract. We have elsewhere adduced evidence which excludes the factors of shock dehydration, and starvation as essential but which emphasizes the importance of the digestion of the blood in the intestinal tract (1, 6)

Attempts have previously been made to evaluate the renal factor in this increase of the blood urea nitrogen Sanguinetti (7) found that three patients with elevated blood urea content following hematemesis were able to excrete a concentration of 30 to 38 grams of urea per liter of urine and believed that this excluded renal insufficiency Christiansen (2) reported normal kidneys at necropsy in a case of hematemesis (due to peptic ulcer) in which there was an abnormally high blood urea concentration. Ingegno (8) emphasized the presence of normal blood pressure, high urine specific gravity and negative urinary find

ings in his patients with elevated blood urea nitrogen. In a fatal case of hematemesis with a blood urea concentration of 216 mgm. per cent Alsted (9) observed a fall in the urea clearance to 15 per cent of normal in the presence of organically normal kidneys He felt that the reduction of renal function was secondary to the lowering of the arterial blood pressure resulting from the hemorrhage. In four other patients displaying less marked azotemia (blood urea values of 42 51, 58 and 59 mgm per cent) there was no reduction of the urea clearance Clausen (10) reported a reduction of urea clearance in three cases though not sufficient to account for the azotemia.⁵

In five patients with increased blood urea content following hematemesis Borst (4) found a normal urea clearance in two and a reduction to 19 47 and 58 per cent respectively in the remaining three. In another group of three patients with shock he found the urea clearance reduced to 10 per cent or less of the normal (3), presumably as a result of the shock. In a series of twelve patients with hematemesis Black (14) recently reported urea clearances of 38 to 47 per cent in four, 59 to 65 per cent in three and 75 to 125 per cent in the remaining five. When the clearances were again determined one week later there was a rise to 87 per cent or more in three of the four cases having an initial value of 38 to 47 per cent and to 65 per cent in the fourth. In the three cases with initial values of 59 to 65 per cent there was no significant change in two and a reduction from 60 to 37 per cent in the third.

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² This work was aided by a grant from the Union Central Life Insurance Company

³ Justin A. Rollman Fellow in Internal Medicine, 1938-1939

⁴ We have observed increase in the blood urea nitrogen in four of eight patients with melena only included in a series of fifty three cases of hematemesis and melena (1)

⁵ According to Chasis and Smith (11) a drop in the urea clearance from 100 to 90 per cent will increase the blood urea by 10 per cent, etc. but an increase of 10 per cent is not ordinarily detectable because of variations in protein intake and variations in urea clearance incidental to oliguria versus diuresis. According to Peters and Van Slyke (12) and Smith (13) it is only when the urea clearance is reduced to 20 per cent of normal that the blood urea nitrogen becomes definitely elevated, irrespective of these other factors.

Black believes that his evidence "is insufficient to establish definitely the mechanism of renal impairment" and that "the fall in urea clearance appears to have some relation to the red cell volume per kilogramme of body weight" Witts (5) believes that the increase in the blood urea is due to the fall in the blood volume and the blood flow through the kidneys at a time when the kidneys have the extra work of excreting a large amount of nitrogen derived from the blood in the intestine

In addition to the urea clearance we have determined the phenol red and inulin clearances in four patients with hematemesis and elevated blood urea nitrogen but without shock or obvious kidney disease. The determinations were made shortly after admission to the Cincinnati General Hospital and were repeated when the blood urea nitrogen was normal

METHODS

The method used was that recommended by Smith *et al* (15), and consisted of a rapid intravenous infusion of normal saline containing inulin and phenol red given for about ten minutes as a priming dose, after which a more dilute solution (usually about 8 per cent) was infused at the rate of 4 cc per minute throughout the period of observation which usually lasted an hour and a half. The urine was collected from the bladder by an indwelling catheter, being allowed to drain into a narrow-necked flask during individual collection periods which varied from ten to twenty minutes. A few minutes before the end of each urine collection period the bladder was washed out with a known amount of distilled water and the wash fluid was expelled by insufflation of air. The urine and wash fluid were combined for analysis, and a preliminary 1:10 dilution was made at once to prevent precipitation of the inulin. Blood samples were usually drawn from the antecubital vein (1) before the infusion was started, (2) after the bladder was first washed just prior to beginning the first urine collection period, (3) after the second collection period, and (4) at the end of the last collection period. Double strength colorless heparin was used to prevent clotting. The blood was centrifuged at once, the plasma separated, and an accurately measured 2 cc. sam-

ple set aside for inulin analysis. Oxalate was added to the rest of the plasma for urea and phenol red determinations. The urea was determined on the plasma and urine by the aeration method of Van Slyke and Cullen (16). The inulin and phenol red determinations were carried out according to the method of Smith and associates (15). All samples were determined in duplicate. The several plasma concentrations were plotted against time and the precise values at the middle of each urine collection period were determined by interpolation. All clearances were corrected to a body surface area of 1.73 square meters at the suggestion of Smith (17).

According to Smith (13), the inulin clearance is a measure of glomerular filtration and in normal man averages about 120 cc per minute, while the phenol red clearance is a measure of tubular function and has an average normal value of 400 cc per minute. The phenol red clearance is an index of renal blood flow. The effective renal blood flow may be calculated approximately from the phenol red clearance and hematocrit by using the average phenol red/diodrast clearance ratio of 0.56 as reported by Smith, Goldring, and Chasis (15), and normally averages about 1400 cc per minute.

CASE STUDIES

Clinical history, case 1

C. F., Number 106697, white male, age 53, entered the Cincinnati General Hospital November 4, 1938. He had been treated for diabetes at this hospital for several years. Eight hours before admission, immediately following his evening meal, he vomited about a pint of bright blood. At 11 p.m. he vomited about one pint of dark, partly clotted blood following which he became dizzy and faint and was sent to the hospital.

The pulse was 104, the blood pressure was 122/60. Free fluid was present in the peritoneal cavity and the spleen was palpable. The left leg had previously been amputated at the mid-thigh.

Hemoglobin 6.6 grams per 100 cc, red blood count 2,600,000, white blood count 9,500. The urine contained a trace of albumin, a two plus test for sugar, and a one plus test for acetone. The stools were tarry and chemical tests for blood were strongly positive. The blood urea nitrogen was 50 mgm. per cent, the blood sugar 351 mgm per cent, and the carbon dioxide combining power 49 volume per cent.

On the second hospital day when the blood urea nitrogen was 37 mgm per cent and the blood pressure was 125/75, kidney function tests were done and found to be

TABLE I

Summary of urea, inulin, and phenol red clearances in case I

Period	Blood pressure	Duration	Urine flow	Plasma			Clearance				Renal blood flow
				Urea nitrogen	Inulin	Phenol red	Urea	Urea	Inulin	Phenol red	
	mm. Hg	min.	cc. per min.	mgm. per 100 cc.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per min.
OBSERVATIONS ON NOVEMBER 5 1938											
1	125/75	14	4.93	37	111	0.91	143	107	140	536	1200
2	125/75	14	4.51	37	109	0.88	119	89	121	460	1038
3	123/75	14	5.31	36	107	0.84	134	101	142	532	1207
4	124/72	20	6.05	35	103	0.80	129	97	139	532	1207

above the average normal (Table I). The patient died on his fourth hospital day at which time the blood urea nitrogen was 17 mgm. per cent. Hepatic cirrhosis and a ruptured esophageal varix were found at necropsy.

Clinical history case 2

H. B., Number 101434 white male age 55 entered the Cincinnati General Hospital August 18 1938, six hours following the vomiting of what he estimated to be a quart of bright blood. He had had three previous episodes of bleeding from the gastro-intestinal tract in the preceding four years. In 1931 he had been found to have a perforated duodenal ulcer.

Upon admission the pulse was 96, the blood pressure 152/80. The hemoglobin was 9.8 grams per 100 cc. red blood count 3,600,000. The urinalysis was normal. His stool was liquid tarry and gave a strongly positive chemical test for blood. The blood urea nitrogen was 24 mgm. per cent, the plasma chlorides 546 mgm. per cent.

Following another bout of hematemesis on the third hospital day the blood urea nitrogen was 40 mgm. per cent. Kidney function tests were done at this time and were repeated ten days later (Table II). The urea and phenol red clearances, when first determined, were moderately decreased; the inulin clearance was normal, and the renal blood flow was diminished. The urea clearance, however, was not sufficiently reduced to account for the elevated blood urea nitrogen. Ten days later the urea clearance was normal although the inulin clearance was definitely reduced and the phenol red clearance less than on the first determination. The discrepancy between the urea and inulin clearances was not explained.

Clinical history case 3

C. K., Number 101015 white male, age 62, entered the Cincinnati General Hospital on August 9 1938. Two days before admission he suddenly experienced vague epigastric discomfort followed by the vomiting of a large

TABLE II

Summary of urea, inulin, and phenol red clearances in case 2

Period	Blood pressure	Duration	Urine flow	Plasma			Clearance				Renal blood flow
				Urea nitrogen	Inulin	Phenol red	Urea	Urea	Inulin	Phenol red	
	mm. Hg	min.	cc. per min.	mgm. per 100 cc.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per min.
OBSERVATIONS ON AUGUST 20, 1938											
1	128/70	15	0.94	35.6	100	1.05	64	34	130	347	735
2	125/72	16	0.67	35.4	100	1.04	64	34	109	327	730
3	124/76	15	0.77	35.4	100	1.02	70	38	128	355	790
4	120/80	15	0.60	35.4	100	1.00	76	41	109	338	752

OBSERVATIONS ON AUGUST 30, 1938											
1	130/80	16	1.62	9.3	122	1.16	81	43	82	273	677
2	128/76	17	1.18	8.6	128	1.20	98	53	80	259	641
3	130/80	17	1.11	8.0	133	1.26	98	53	83	271	672
4	142/82	17	1.23	8.7	150	1.33	101	55	76	249	618

amount of clotted blood. On the day of admission he vomited dark blood several times.

The pulse was 88. The blood pressure was 90/40 but rose soon afterwards to 118/50. The abdomen was negative.

TABLE III

Summary of urea, inulin, and phenol red clearances in case 3

Period	Blood pressure	Duration	Urine flow	Plasma			Clearance				Renal blood flow
				Urea nitrogen	Inulin	Phenol red	Urea	Urea	Inulin	Phenol red	
	mm. Hg	min.	cc. per min.	mgm. per 100 cc.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per 1.73 sq m. per min.	cc. per min.
OBSERVATIONS ON AUGUST 9 1938											
1	118/50	12	0.58	38	136	1.01	75	40	48	228	486
2	120/55	12	0.58	38	143	1.02	55	31	48	221	472
3	120/55	13	0.69	38	150	1.03	57	30	55	233	498
4	124/58	11	0.72	39	150	1.05	59	33	54	233	498
5	124/58	10	0.70	40	150	1.05	45	23	51	235	479

OBSERVATIONS ON AUGUST 23 1938											
1	128/50	15	2.0	12	105	0.9	43	32	74	256	542
2	128/50	15	2.7	12	110	1.0	93	70	80	252	580
3	128/50	15	1.9	13	150	1.0	58	44	71	270	506
4	128/50	15	1.4	13	120	1.0	54	28	70	232	530
5	128/50	11	1.4	14	140	1.1	48	26	67	256	579

The hemoglobin was 5.8 grams per 100 cc. red blood count 1,800,000. The urine was normal. The stools were tarry and the guaiac test for blood was strongly positive. The blood urea nitrogen on admission was 40

mgm. per cent, carbon dioxide combining power 52 volume per cent, and the plasma chloride 564 mgm. per cent.

Kidney function tests were done shortly after admission and again on the fourteenth hospital day when the blood urea nitrogen was 13 mgm per cent (Table III). There was a reduction of all the clearances and a decrease in the effective renal blood flow. The decrease in the urea clearance, however, was not sufficient to account for the elevated blood urea content. Fourteen days later there was no significant change in renal function or in the effective renal blood flow although the blood urea nitrogen was normal.

Clinical history, case 4

W B, Number 106702, white male, age 57, entered the Cincinnati General Hospital on November 4, 1938. He had had two episodes of hematemesis in the past and had been found to have a hypertrophic gastritis. The night before admission he suddenly vomited a large amount of blood. This was followed by the passage of three liquid dark red stools. Shortly after admission he again vomited a large amount of dark clotted blood.

The pulse was 100, his blood pressure 115/70. The hemoglobin was 88 grams per 100 cc., red blood count 2,700,000. The urinalysis was normal. The stools were tarry and gave a strongly positive test for occult blood. The blood urea nitrogen was 41 mgm per cent and the carbon dioxide combining power of the blood 56 volume per cent.

TABLE IV

Summary of urea, inulin, and phenol red clearances in case 4

Period	Blood pressure	Duration	Urine flow	Plasma			Clearance				Renal blood flow
				Urea nitrogen	Inulin	Phenol red	Urea	Urea	Inulin	Phenol red	
		min utes	cc per min ute	mgm per 100 cc			per cent of normal	cc per 1.73 sq m per minute			cc per min- ute
OBSERVATIONS ON NOVEMBER 5 1938											
1	120/68	20	3.25	63.9	140	1.06	50	37	82.4	356	836
2	118/70	16	3.81	64.0	158	1.15	53	40	74.0	302	701
3	122/70	12	4.00	64.1	193	1.31	59	46	78.2	377	888
OBSERVATIONS ON NOVEMBER 8 1938											
1	148/70	14	1.7	14.6	129	0.74	47	31	100.0	355	790
2	145/70	15	1.8	14.8	137	0.79	42	33	95.0	371	829
3	145/70	11	2.0	15.2	145	0.84	67	52	97.0	421	989
4	140/70	10	2.4	15.7	152	0.88	73	54	99.0	462	1030

Four hours after admission the blood urea nitrogen had risen to 64 mgm. per cent. Kidney function studies were done at this time and again three days later when his blood urea nitrogen was 15 mgm per cent. The results are shown in Table IV. While there was a reduction of all the clearances and of the effective renal blood flow, the decrease in the urea clearance was not

sufficient to account for the elevated blood urea nitrogen. Three days later, when the blood urea nitrogen was normal, there was no significant difference in renal function or in the effective renal blood flow.

According to Peters and Van Slyke (12), any reduction of the urea clearance is due either to decreased renal blood flow or to less complete extraction of urea from the blood. It is possible that reduction of renal blood flow played a rôle in reducing the clearances in three of our cases but to an extent not sufficient to account for the elevated blood urea nitrogen.

It has been suggested that toxic products arising from the decomposition of the blood which is liberated in the intestines following hematemesis and melena may lead to impairment of kidney function and thus account for elevation of the blood urea nitrogen (18). To exclude such a factor, the kidney function tests were carried out in five individuals two days before and eighteen hours after intragastric administration of 2,000 cc of citrated blood⁶. The five subjects included Cases 2 and 3 and, in addition, three males (free

TABLE V

Renal function before and eighteen hours after beginning intragastric administration of 2,000 cc of blood

Case number	Before blood administration				After blood administration			
	Blood urea nitrogen	Clearances			Blood urea nitrogen	Clearances		
		Urea	Inulin	Phenol red		Urea	Inulin	Phenol red
	mgm per cent	per cent of normal	cc per 1.73 sq m per minute		mgm per cent	per cent of normal	cc per 1.73 sq m per minute	
2	13	72	87	283	37	70	83	268
3	13	54	74	239	51	54	76	252
5	11	103	99	281	35	74	122	337
6	12	88	106	363	26	81	116	339
7	14	113	117	316	54	108	128	348

of obvious kidney disease) aged 19, 29, and 50 respectively. Two of the three had had a recent hematemesis, while the third was suffering from optic atrophy. The results show that no significant change in renal function occurred (Table V).

⁶ The blood, which was previously stored in the blood bank for three weeks, was given by stomach tube in doses of 700, 700, and 600 cc at four-hour intervals. Following introduction of the blood, there was a distinct rise in the blood urea nitrogen reaching a maximum twenty hours after the first dose was given (6).

SUMMARY AND CONCLUSION

The urea, inulin and phenol red clearances may be either normal or reduced in the presence of the increased blood urea nitrogen content which follows hematemesis. The reduction in the urea clearance may be due to the decreased renal blood flow but is insufficient to account for the increased blood urea content and persists in spite of the return of the blood urea nitrogen to normal.

The elevation of the blood urea nitrogen content which follows hematemesis in the absence of shock is not due to impairment of kidney function.

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IMMUNITY IN DIABETES III RELATION OF TISSUE GLYCOGEN AND BLOOD CHEMISTRY TO BACTERIAL DISSEMINATION, ANTIBODY FORMATION AND SURVIVAL AFTER INFECTION IN DIABETES

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In former publications from this laboratory (1, 2) it was shown that diabetic patients were unable to form agglutinin to as high a titer as normal controls following inoculation with a B. typhosus vaccine given according to the standard technique. It was further demonstrated that the bactericidal power of the blood from diabetic patients was definitely less than that of normal controls when tested against six pathogenic organisms. This failure was found to be due to the amboceptor, as the blood from the diabetics contained complement in an amount equal to that of non-diabetic controls. At the same time it was shown that the smallest amount of agglutinin was formed by those diabetics who were in the most unsatisfactory metabolic balance. Rabbits which had been kept on a low food intake for several weeks before the inoculation with vaccine always showed a similarly diminished ability to form agglutinin with some alteration in metabolic balance as evidenced by a marked loss in body weight. From these investigations it appeared that at least a part of the commonly recognized susceptibility of diabetics to infection might be concerned with their decreased power to form immune bodies as compared with normal individuals.

A large part of the previous work on the resistance of the body to disease has dealt only with the immunological aspect. In addition to the variations in antibacterial activity there are, of course many chemical and physical alterations in the tissues and fluids of the host which may have an influence on the ability of the body cells to react to such bacterial products as are not overcome by the antibacterial forces of the body. The bacteria themselves have a variable ability to attack the host with their toxins and to protect

themselves by such means as their capsules. Thus infection with its accompanying inflammation would appear to consist of an interaction of the offensive and defensive forces of both the host and the infecting agent.

In this report are presented the results of some further observations and experiments on the formation of antibodies and the resistance to infection in diabetic patients and experimental animals. In the latter an attempt has been made to reproduce certain of the factors which are commonly present in the diabetic with a view to determining their influence on the ability of the body to resist infection. Among these factors are the amount of sugar of the blood, the total protein, albumin, globulin, and cholesterol of the serum and glycogen of the tissues. These factors have been studied in connection with (1) the ability to form antibodies in both diabetic patients and experimental animals, (2) the survival time after intravenous inoculation of animals with bacteria, (3) the spread of bacteria throughout the body from an experimentally inoculated focus and (4) the ability of the blood to destroy the bacteria *in vivo*.

In experiments on the formation of antibody B typhosus vaccine was used according to the standard technique. In all other experiments suspensions in normal saline of an 18-hour broth culture of staphylococcus aureus were used. This strain of staphylococcus aureus, which has been used throughout the entire investigation, was isolated from the blood of a fatal case of bacteremia, and has been maintained for the past 3 years by semi-weekly transplants on plain agar plates. Only smooth colonies were fished for transplant so that few rough colonies appear in the culture.

The blood sugar was determined by the Benedict method, serum cholesterol by Bloor's method, and serum protein and serum albumin by Pregl's modification of the Kjeldahl. Serum globulin was taken as the difference between the total protein and the albumin. Glycogen of the tissue was determined by a modification of Pfäuger's method.

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The agglutinative titer was measured by Dreyer's macroscopic method, using a formalized antigen, readings being made after 2 hours in the water bath at 55° C. followed by 18 hours' refrigeration at 6° C.

Quantitative determination of bacteria in blood was made by plating one half cubic centimeter of blood in plain agar by standard bacteriological technique. Tissues were ground with sterile sand and plated in plain agar plates after proper dilutions with broth according to standard technique. Control cultures of bacteria-free blood and tissues were made to check the sterility of these procedures

In an attempt to determine the influence of the amount of sugar in the blood and the amount of glycogen in the tissues on the survival time of experimental animals following an intravenous inoculation with bacteria, the following investigations were undertaken. Normal, adult rabbits weighing about 3 kgm were used. Each rabbit was given intravenously from 1 to 7 cc of an 18-hour broth culture of staphylococcus aureus. Different amounts of bacterial suspension were given in different experiments, but all the animals in each experiment received the same amount. At intervals after the inoculation blood was withdrawn from an ear vein for culture and sugar determination. For the sake of clarity the experiments will be described in three groups.

In Group I the rabbits were given glucose intravenously during the course of the bacteremia, in Group II they were given epinephrine subcutaneously during the course of the bacteremia, and in Group III they were given glucose intravenously previous to the inoculation with bacteria.

In Group I and II blood was withdrawn from an ear vein for culture and determination of sugar 3 or 4 times at intervals of 4 hours after the inoculation with bacteria. The animals were used in the same order for the inoculation and each successive bleeding, so that the elapsed time between successive determinations was as nearly equal as it could be made, and in each rabbit did not vary more than 10 minutes from the average for the whole group. The animals seldom showed any clinical evidence of the septicemia for from 6 to 8 hours after the inoculation. After this, however, some began to show symptoms of the infection and 11 or more hours after inoculation death occurred.

Group I In 8 experiments 22 rabbits were given glucose intravenously and 13 controls were

given an equal amount of physiological saline solution. The results of the blood cultures on these animals are shown in Figure 1. They are given as the ratio between the number of bacteria in 0.5 cc of blood from the rabbits which had received sugar, and the number in the blood from the control rabbits used in the same experiment, and are plotted logarithmically. The comparisons were made only between the cultures taken an equal number of hours after the injection of the bacteria. If only one control rabbit was used with several rabbits receiving sugar, the blood cultures of the latter were each compared with the single control. If more than one control rabbit was used, an average of the number of bacteria in the blood cultures of all the control rabbits was used for comparison with the number of bacteria in the blood of each rabbit receiving sugar. These ratios are grouped according to the sugar in the blood of the glucose-treated rabbits taken at the same time as the blood culture. In the first sub-group are shown those in which the blood sugar was below 140 mgm, in the second sub-group those with blood sugar between 140 and 200 mgm, and in the third sub-group those with a blood sugar over 200 mgm per 100 ml of blood at the time the culture was taken.

It is evident that there is no significant difference in the number of bacteria in the blood of those animals given intravenous glucose during bacteremia, whether their blood glucose was normal or high, and the controls with a normal blood sugar.

Group II In 3 experiments 7 rabbits were given epinephrine and 5 were used as controls. The bacteria were given intravenously 1 hour after the first injection of epinephrine while the blood sugar was above 200 mgm per 100 ml of blood. Blood for culture and sugar determination was withdrawn as in the experiments of Group I.

The results are also shown in Figure 1. It is apparent that there is no significant difference between the number of bacteria in the blood of the control rabbits and that of the rabbits with hyperglycemia produced by injections of epinephrine.

Group III In 4 experiments in which 16 rabbits were given glucose and 9 were used as controls, 7 intravenous injections of from 2.5 to 5

grams of glucose were given at hourly intervals, followed after the seventh injection by the inoculation with bacteria as previously described. No glucose was given after the inoculation. Blood for culture and sugar determination was withdrawn 15 hours later.

The results of these determinations are also shown in Figure 1. It is evident that the injection of glucose before the bacteria were given had no significant influence on the number of bacteria in the blood.

In order to determine the effect of the bac-

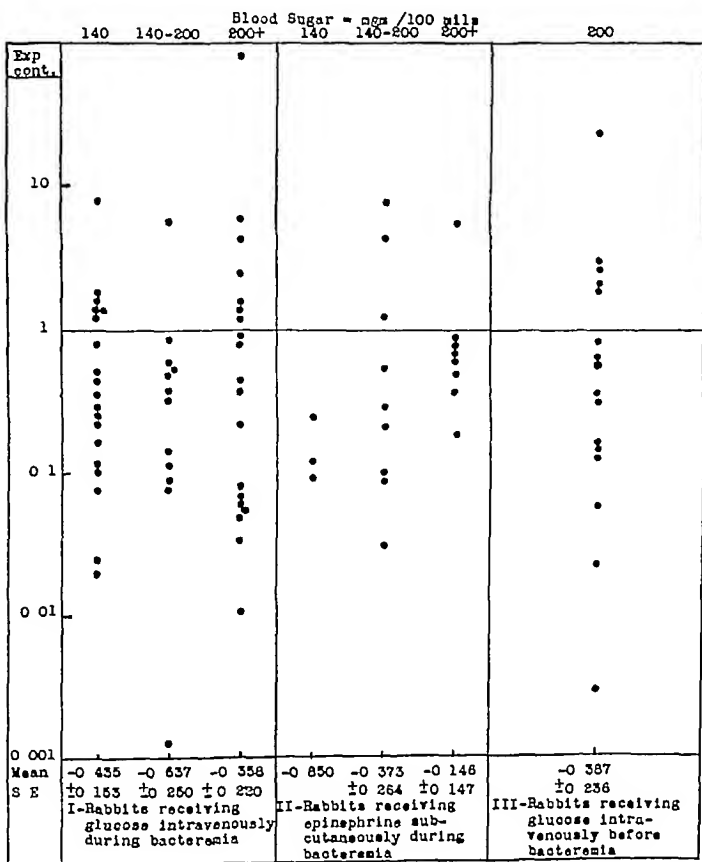


FIG. 1. RATIO OF BACTERIAL COUNT IN EXPERIMENTAL ANIMALS WITH RAISED BLOOD SUGAR TO BACTERIAL COUNT IN CONTROL ANIMALS BLED AT SAME INTERVAL AFTER INTRAVENOUS INJECTION OF BACTERIA

Blood sugar indicates the values found in experimental animals at moment when bled for blood culture. Mean = $\frac{\log \text{exp.} - \log \text{cont.}}{n}$ where log exp. and log cont. are the logarithms (base 10) of the bacterial counts. S.E. = standard error of mean.

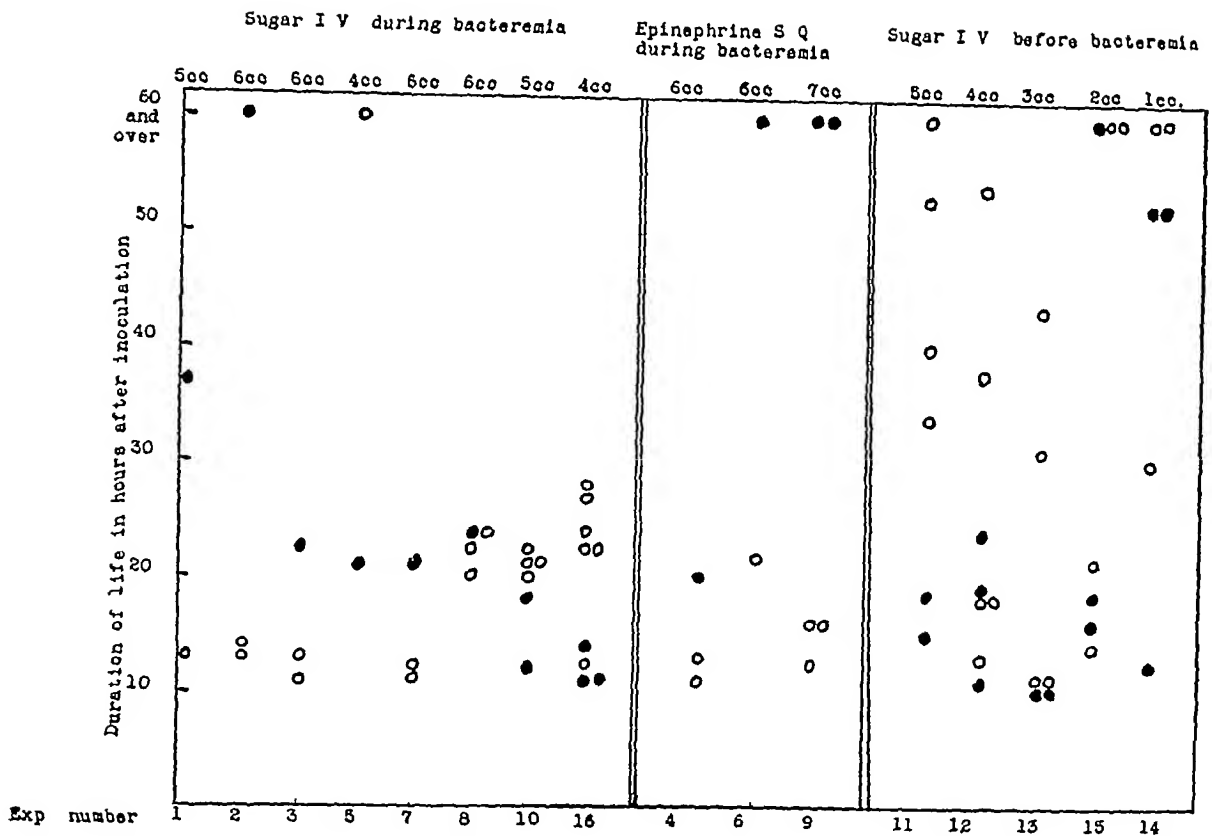


FIG 2. DURATION OF LIFE IN HOURS AFTER INOCULATION WITH BACTERIA IN EXPERIMENTS NUMBERS 1 TO 16
 O = rabbits receiving glucose or epinephrine. ● = control rabbits

teremia on the rabbits, their survival time after inoculation was studied. The results are shown in Figure 2. The difference in number of bacteria injected makes a comparison between animals in different experiments difficult. For this reason numbers 1, 5, 10, 16, 11 and 12, in which 4 or 5 cc. of the broth culture were used, were selected for analysis. In these experiments 12 rabbits had been given glucose during the bacteremia, 9 had been given glucose before the bacteremia, and 12 were controls. Table I shows the average duration of life in hours of the animals in these three groups.

Glucose appears to have no significant influence on the duration of life when given during the bacteremia, but when given for several hours previous to injection of the bacteria there is a greater increase in survival time, although the significance of this is not clear.

As approximately the same amount of bacteria had been injected into all these rabbits, there was no significant variation in the results of the culture, it would ap-

TABLE I

Survival time of rabbits without glucose and with glucose during or before the bacteremia produced by intravenous injection of staphylococcus aureus

	Hours of survival
Control rabbits (12)	17±1.8
Rabbits given I V glucose during bacteremia (12)	21±4.7
Rabbits given I V glucose before bacteremia (9)	34±6.2

pear that the longer duration of life may have been due to resistance to the action of the bacteria provided by the glucose given before the bacteremia.

In view of these experiments, which suggest that glucose, given intravenously, may increase the resistance of the body to the bacteria, the glycogen content of the liver was determined as evidence of the glycogen stores of the body. The averages of these determinations are shown in Table II. The tissue was taken under nembutal

anesthesia when it was evident that the animal would die soon. The greatly increased glycogen in the liver of the rabbits receiving glucose intravenously as shown in this table is correlated with the longer duration of life in the rabbits in Group III shown in Table I.

In view of these results it was decided to continue this work with depancreatized cats. It was thought that in this way an experimental diabetes could provide a more prolonged hyperglycemia than was obtained in the rabbits and certain other alterations in the chemistry of the body commonly found in diabetic patients.

TABLE II

Per cent of liver glycogen in rabbits which had received injections of bacteria with glucose intravenously or epinephrine subcutaneously

	Per cent of liver glycogen
Control rabbits	0.63±0.244
Rabbits receiving epinephrine subcutaneously	0.56±0.237
Rabbits receiving glucose intravenously	6.38±1.27

Normal cats about 2.5 kgm. in weight were completely depancreatized and maintained for from 3 to 60 days on a diet of fish, beef liver and pancreas. Insulin was given subcutaneously twice daily in different amounts, depending on the diet. No attempt was made to maintain normal blood sugar. In order to vary the nutritive condition of the animals the diets given varied from a normal diet to one slightly above basal requirements. Some cats refused food for several days before the experiments in which they were used.

Thirteen experiments were done in each of which 1 normal and 2 depancreatized cats were generally used. At the beginning of each experiment, under nembutal anesthesia, blood was taken from the inferior vena cava for determination of sugar, serum cholesterol and serum protein, and tissue from liver and muscle for glycogen. One cc. of staphylococcus aureus suspension per kilo of body weight was then injected into the inferior vena cava. For each experiment an 18-hour broth culture was centrifuged and the bacteria resuspended in physiological saline solution so that each cubic centimeter contained 2,000 million bacteria. At 24 and 48 hours after the inoculation such animals as were still living were again anesthetized and blood and tissue removed as already described. Blood was removed at these times for culture also. One-half cc. of blood was plated with agar in Petri dishes according to standard blood culture technique. After 48 hours incubation at 37° C. the colonies in each plate were counted.

In order to determine the effect of the operative proce-

dures on the animals 3 normal and 3 depancreatized cats were operated on from 2 to 4 times without inoculation with bacteria. One of these cats became infected and died. The other 5 lived. It would appear therefore, that as both the depancreatized and the normal control cats were operated on in the same manner the operative procedures were not a significant factor as between the two groups.

For purposes of comparison the depancreatized and the normal control cats were divided into four groups according to the survival time after inoculation with bacteria. In the first group 6 depancreatized animals lived less than 18 hours after the inoculation. No normal cats died within this time. In the second group 5 depancreatized cats lived longer than 18 hours and less than 30 hours. One control animal died within these limits. In the third group 13 depancreatized cats survived to more than 30 hours and less than 80 hours. Eleven normal cats died within these limits after receiving the bacteria. The fourth group comprised those animals which recovered entirely from the infection. In this group there were no depancreatized cats, and 4 control cats.

TABLE III

Duration of life in hours after inoculation with bacteria, and glycogen of liver in depancreatized and normal cats

	Less than 18 hours	18 to 30 hours	30 to 80 hours	Survived infection
	per cent	per cent	per cent	per cent
Depancreatized cats.	(8) 0.43±0.14	(3) 1.61±0.31	(12) 2.74±0.26	None
Normal cats.	None	(1) 1.11	(11) 2.28±0.58	(4) 2.05±0.53

Number of animals in each group is shown in parentheses, liver glycogen in per cent.

The survival time and the glycogen of the liver of these cats at the time of inoculation with bacteria are shown in Table III. It would appear from these experiments that as shown in this table those depancreatized animals which had more glycogen in the liver at the time of inoculation survived a longer time after the inoculation with bacteria.

The amount of glycogen in the muscles of these animals at the time of inoculation is shown in Table IV. It is apparent that there is no significant correlation between the survival of the animals and the glycogen of the muscle at the time of

TABLE IV

Duration of life in hours after inoculation with bacteria, and glycogen of muscle in depancreatized and normal cats

	Less than 18 hours	18 to 30 hours	30 to 80 hours	Survived infection
	per cent	per cent	per cent	per cent
Depancreatized cats	(6) 0.90 ± 0.18	(5) 0.99 ± 0.15	(13) 0.99 ± 0.08	None
Normal cats	None	(1) 0.97	(11) 0.98 ± 0.08	(4) 0.90 ± 0.17

inoculation in either the depancreatized cats or the controls

The loss in the amount of glycogen in the liver during the 24 hours after inoculation of the cats which died is shown in Table V. The cats in Group I did not, of course, survive long enough to have a second operation.

TABLE V

Percentage loss of liver glycogen of depancreatized and normal cats during 24 hours following their inoculation with bacteria

	Group II 18 to 30 hours	Group III 30 to 80 hours	Group IV survived infection
	per cent	per cent	per cent
Depancreatized cats	52 ± 21 (3 cats)	46 ± 9.7	(No cats)
Normal cats	61 (1 cat)	46 ± 9.9	41 ± 11.2

It is evident that there is not a significant difference in the percentile decrease of glycogen in the liver in depancreatized cats as compared with normal cats. In Group II, in which the animals lived for from 18 to 30 hours after the inoculation, only 3 depancreatized cats lived over 24 hours and were available for a second determination of glycogen.

The change in the glycogen of the muscle was found to be highly variable and very different from that of the liver during the 24 hours after inoculation. Of the depancreatized cats in Group III, which lived from 30 to 80 hours after the inoculation, 4 had an increase of from 13 to 28 per cent, while 7 cats lost from 7 to 74 per cent of the amount of glycogen present 24 hours earlier. In the normal cats 5 showed an increase in muscle glycogen of from 35 to 87 per cent, while 6 showed a decrease of from 8 to 79 per cent. There was thus no constant reaction of the muscle glycogen to the infection nor was there any cor-

relation between it and the duration of life after inoculation. In those animals in which there was an increase in percentile muscle glycogen there was usually a greater decrease in the percentile glycogen in the liver. With such changes in distribution of the glycogen in the body no inferences can be drawn as to total body glycogen.

The number of bacteria in the blood 24 hours after inoculation varied greatly in both the depancreatized and in the normal animals. There was, however, no significant difference in this respect between the two groups. Table VI shows the results of these determinations.

TABLE VI

Blood sugar and number of bacteria per 0.5 cc of blood, cultured 24 hours after inoculation with bacteria, in depancreatized and normal cats which died 18 to 80 hours after inoculation

	Blood sugar	Number of bacteria per 0.5 cc of blood
	mgm	
Depancreatized cats	Over 200	2226 ± 1034
	Under 200	1009 ± 980
Normal cats	Over 140	363 ± 300
	Under 140	1279 ± 1100

All cultures of the blood at the end of the 24-, 48- and 72-hour periods contained bacteria. In those animals which died there was no correlation between the number of bacteria in the blood at these times and the duration of life after inoculation with bacteria. In the 4 normal cats which survived the infection there appeared to be a definitely smaller number of bacteria per 0.5 cc of blood at these times. Cultures on these animals made at the end of 24 hours contained 96 ± 43 bacteria per 0.5 cc of blood. A study of the sugar and of the number of bacteria in the blood of the depancreatized animals shows no significant correlation between them.

The cholesterol of the serum in the depancreatized cats varied from 65 to 277 mgm per 100 ml of blood and showed no correlation with the hours of survival after inoculation with bacteria. In the normal cats the cholesterol of the serum was in general slightly lower than in the depancreatized cats, varying from 87 to 185 mgm per 100 ml of blood. No correlation appeared, however, between the survival time and the cholesterol of

the blood in these animals either in the individuals or in the averages of the groups

The total protein in the serum of the depancreatized cats varied between 6.07 and 8.65 per cent and of the normal cats from 6.54 to 8.15 per cent. No significant correlation was found in these animals between the protein of the serum and either the duration of life after inoculation with bacteria or the number of bacteria in the blood.

In the foregoing experiments in which the bacteria were given intravenously it is recognized that this method of inoculation is not above criticism, in that it does not reproduce infection in the experimental animals in the manner in which it occurs in the patient. Seldom, if ever does the blood stream receive bacteria in the large numbers given in those experiments. For that reason it was decided to continue the investigation by giving a much smaller number of bacteria intradermally and after a certain number of hours to examine the blood and various tissues in order to determine whether the bacteria in the skin were destroyed and whether there might be found in the normal and depancreatized animals any significant difference in the frequency with which certain other organs of the body had become infected from this focus. In this investigation cats of the same weight as those used in the earlier experiments were depancreatized and maintained in the same manner.

In from 3 to 65 days after pancreatectomy 0.1 cc. of a suspension of *staphylococcus aureus* made from an 18-hour broth culture was injected into the skin of the abdomen of 2 depancreatized cats and 1 normal cat. The suspension was made so that 0.1 cc. contained 8 million bacteria. After 24 hours the animals were anesthetized with nembutal and the inoculated area of skin as well as the blood, liver and spleen, was cultured by standard bacteriological technique. The cultures were made quantitatively so that the results showed the number of bacteria in 0.5 cc. of blood, 1 gram of liver half of the spleen, and the entire inoculated area of the skin. In order to control the sterility of the method, a number of cultures of normal skin were made. No bacteria were recovered in these cultures. An adjacent piece of liver spleen a piece of skin from the side of the body opposite the area of inoculation and symmetrically located and a piece of muscle were taken for determination of the glycogen content. Blood was also taken for sugar and for total protein albumin, and cholesterol of the serum. The serum globulin was taken as the difference between

TABLE VII

Percentage of depancreatized and control cats in the organs of which bacteria were found 24 hours after an intradermal inoculation of staphylococcus aureus

	Normal	Depancreatized
Number of animals inoculated	38	58
Per cent with bacteria in liver	35	77
Per cent with bacteria in spleen	29	81
Per cent with bacteria in blood	16	19
Per cent with bacteria in skin	76	78

the total protein and the albumin. Thirty-eight normal and 58 depancreatized cats were studied in this manner.

Table VII shows the results of the cultures in these animals. As will be noted there is no difference between the normal and the depancreatized cats in the frequency with which the blood and the site of inoculation in the skin contain bacteria. However the liver and spleen of the depancreatized animals contained bacteria with definitely greater frequency than those of the normal controls. Furthermore, as shown in Table VIII the liver and spleen of the depancreatized

TABLE VIII

Bacteria in liver and spleen in normal and depancreatized cats

LIVER		
24 normal cats.	35 per cent contained bacteria	27 bacteria per gram of tissue
58 depancreatized cats	77 per cent contained bacteria	500 bacteria per gram of tissue
SPLEEN		
31 normal cats.	29 per cent contained bacteria	21 bacteria per spleen
53 depancreatized cats	81 per cent contained bacteria	320 bacteria per spleen

animals in which bacteria were present contained a much larger number of organisms than did those organs in the normal controls which contained bacteria. No significant difference is found in the chemistry of the blood and tissues in the normal and depancreatized animals. Table IX shows an analysis of the amount of glycogen in the liver and skin of the sugar of the blood and of the cholesterol, total protein albumin and globulin of the serum of the depancreatized cats. It was not possible to find any significant correlation between the liver glycogen sugar cholesterol total protein or albumin and the presence of bac

as high a titer as normal controls. In this way it was thought possible to reproduce at least some of the factors commonly found in the diabetic patient and to ascertain, if possible, the influence of these factors on antibody formation. To this end depancreatized and normal cats were given B typhosus vaccine and the sugar of the blood, the agglutinative titer, total protein, albumin, globulin and cholesterol of the serum, and glycogen of the liver and of the muscle were determined.

Two series of experiments were carried out. In both series normal cats weighing about 2.5 kgm were depancreatized and, after recovery from the operation, were placed on a diet consisting of pancreas, fish and liver. Regular insulin or protamine zinc insulin was given in sufficient quantity to maintain the animals in as nearly normal a condition as possible. The controls were normal cats of about the same weight maintained throughout the duration of the experiments on a diet of fish and beef heart. The cats in the first series of experiments were given a diet of two and one half times the basal requirement with more insulin, while those in the second series received 50 per cent more than the basal requirement of food and less insulin than those of the first series.

In each experiment 3 control cats and 3 cats depancreatized from 2 to 60 days previously, were generally used. All were given 0.1 cc of B typhosus vaccine intravenously. Four depancreatized cats and 1 control cat died less than 24 hours after receiving vaccine.

In the first series of experiments a total of 6

depancreatized and 10 normal cats which survived the inoculation were anesthetized with nembutal 7 days after inoculation, and blood was taken for determination of the agglutinative titer. Table XII shows the agglutinative titer of these animals. It is apparent from these figures that there is no significant difference in the agglutinative titer of the serum of the normal and of the depancreatized animals.

A second series of experiments was done on 6 normal and 12 depancreatized cats. All procedures were the same as those used in the first series except that a different lot of B typhosus vaccine was used, and the animals were given a lower caloric diet and less insulin. The agglutinative titer of the blood of these animals taken on the 7th day is shown in Table XII. It will be noted that on this day there appears to be a slightly higher titer in the depancreatized than in the normal cats of the second series of experiments.

Table XIII gives the results of the other examinations of the blood and tissues in these animals. The table shows that, although the serum cholesterol is generally lower in the depancreatized animals, there is no correlation between this and the agglutinative titer in the individual animals. However, of the 4 cats with the lowest titer, 2 had no chemical analyses. Of the other 2, both had much lower liver glycogen than any of the normal or other depancreatized cats, and 1 had lower serum protein and serum globulin than the other cats. Examination of the individual protocols indicates that these 4 cats were less well nourished, either through refusal of food or inadequate insulin, than any of the other depancreatized cats in the table. Their average daily intake of meat during the experiments varied between 39 and 69 grams while the other depancreatized cats took between 97 and 130 grams of meat daily.

The elapsed time between the pancreatectomy and the inoculation appears to have no influence on the formation of agglutinin.

It is apparent from the above experiments that depancreatized cats, as compared with normal controls, may lose the ability to form antibody in the blood when their nutrition is impaired. They may thus be compared to the diabetic pa-

TABLE XII

Agglutinative titer of depancreatized and normal cats

1ST SERIES								
Agglutinative titer	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Died
Normal cats	1	2	3	0	2	1	1	1
Depancreatized cats	0	0	1	1	1	2	1	3

2ND SERIES									
Agglutinative titer	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Died
Normal cats	1	2	2	1	0	0	0	0	0
Depancreatized cats	0	1	1	3	1	3	2	0	1

TABLE XIII

Chemical analyses of the blood and tissue of depancreatized and normal cats in which the agglutinin titer is shown in Table XII (2nd series)

NORMAL CATS

Cat number	Agglutinin titer	Sugar	Cholesterol	Protein	Albumin	Globulin	Liver glycogen	Skin glycogen
		mgm.	mgm.	per cent	per cent	per cent	per cent	per cent
718	1/220	86-101	188	7.20	3.20	4.00	0.58	0.07
719	1/160	85-154	194	5.90	3.72	3.18	0.54	0.08
714	1/160	50-84	185	5.00	1.12	4.78	1.17	0.19
713	1/160	80-98	182	7.14	3.01	4.13	1.18	0.18
711	1/80	50-50	263	0.50	2.53	0.75	0.53	0.17
713	1/90	65-85	125	8.44	3.23	0.73	0.86	0.12
Average			186	7.34	2.52	4.73	0.94 ±0.08	0.13

DEPANCREATIZED CATS

Cat number	Agglutinin titer	Sugar	Cholesterol	Protein	Albumin	Globulin	Liver glycogen	Skin glycogen	Urine sugar	Days after operation
		mgm.	mgm.	per cent	per cent	per cent	per cent	per cent		
681	1/2500	376-291	187	8.23	3.17	5.06	0.63	0.16	++++	20
693	1/2500	93-250	53	8.72	2.22	0.40			++++	24
686	1/640	187-310	77	9.58	3.27	6.30			± to 0	29
680	1/640	84-218		8.45					++++	36
715	1/640	281-258	280	8.00	2.25	5.75				2
692	1/230	292-344	147	6.40	2.40	4.00	0.64	0.19	++++	43
684	1/230	181-370	90	7.38	3.96	3.42	0.66	0.21	++++	36
703	1/230	182-192	118	7.14	3.51	4.60	1.49	0.14	± to +++++	28
716	1/80	300-344	69	5.10	2.28	2.82	0.35	0.05	++++	3
717	1/80	182-340	67	8.70	2.66	4.14	0.15	0.00	++++	2
708	1/40	—	Died 7th day						0 to +++++	18
697	1/160	82-206	Died 7th day						± to +++++	34
Average			115	7.57	2.74	4.76	0.68 ±0.19	0.12		

tients and the underfed rabbits reported in previous communications. Of course little is known concerning the antibody response to a bacterial antigen in cats. They were selected for this work however because at the time it had hardly been demonstrated that the pancreas may be safely removed from rabbits the animals on which most of the antigen antibody work has been done. It appears that the cats vary considerably in their response to the vaccine though, on the

whole it would seem that they have less capacity than some other animals to form antibodies following the injection of vaccine.

The fact must not be overlooked that, while we do produce in the depancreatized animal kept under partial control with insulin some of the alterations in blood and tissue chemistry commonly found in the diabetic we do not produce diabetes mellitus as a definite clinical entity. The long duration of the diabetic state in the patient, as compared with the relatively short time in these experiments between pancreatectomy and inoculation of the animals with the vaccine may have a significant influence on antibody formation. Furthermore some endocrine or other metabolic alteration perhaps present in the patient, but not found in the animals under the conditions of these experiments may account for the difference in the reactions in the two groups.

DISCUSSION

Without attempting to make a final report now, it is, nevertheless, desirable to indicate certain conclusions which may properly be drawn from the foregoing experiments. It would seem that the impaired ability to build agglutinins noted in the diabetic patient is possibly associated with long continuation of the pathologic state. In all of these experiments variations in the blood chemistry, some of which are commonly found in the diabetic patient appear with the exception perhaps of globulin to have no effect on the formation of agglutinins. The survival after intravenous inoculation with bacteria, the growth of bacteria in the blood or the dissemination of bacteria to various organs from a primary focus in the skin. There is a significantly smaller amount of serum globulin in the blood of those depancreatized cats in which the liver or spleen showed bacteria 24 hours after the intradermal inoculation with bacteria. The decreased ability in the patient to form agglutinins may perhaps be caused by an altered nutritional state similar to that in the underfed rabbits and the last 4 of the depancreatized cats in Table XIII.

The ability of the experimental animal to survive after a bacterial infection appears to be definitely increased by the presence of a larger amount of glycogen in the liver or by some meta-

bolic change which accompanies this increased liver glycogen. On the other hand, this larger amount of glycogen does not appear to increase the ability of the individual tissues or organs to inhibit the growth of bacteria coming to them from a primary focus in the skin.

In these experiments it was evident that depancreatized cats exhibited definitely greater frequency of dissemination of bacteria from a focus in the skin to the liver or spleen. These observations in the animals under the conditions of these experiments accord fully with the clinical experience that, in general, the diabetic is less able than the normal person to control adequately even a mild infection in the skin.

Whether this inability to curb the bacteria resides in the tissues of the primary focus or in the organs to which the bacteria are disseminated, or both, is as yet unknown. The abnormal condition which allows the bacteria to invade the organs of the diabetic, as well as of the depancreatized animal, may be in the tissue in which the primary focus occurs. It is suggested from the above experiments that the percentile amount of glycogen in the skin reflects this alteration in the tissue.

CONCLUSIONS

1 A significant correlation is shown to exist in normal rabbits and depancreatized cats between the percentile amount of glycogen in the liver and the survival time after intravenous inoculation with bacteria.

2 A lowered nutritional state, accompanied by decreased liver glycogen, is shown to exist in depancreatized cats in which a low agglutinative titer is found after injection of *B. typhosus* vaccine.

3 Alterations in the blood of these animals, such as are commonly found in diabetic patients, do not appear to influence the survival time or the ability to form agglutinins.

4 The organs of depancreatized cats 24 hours after an intradermal inoculation with bacteria show the presence of these bacteria with greater frequency than do the organs of normal controls. Alterations in the sugar of the blood and of the cholesterol, protein, albumin and globulin in the serum and glycogen in the liver do not appear to influence this dissemination of bacteria from a focus.

5 Acidosis appears to increase the frequency with which this dissemination of bacteria occurs.

6 The percentile amount of glycogen in the skin of depancreatized cats shows a suggestive, though not clearly significant, correlation with this dissemination of bacteria from a skin focus to the organs of the body.

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THE URINARY EXCRETION OF RIBOFLAVIN FLUOROMETRIC METHODS FOR ITS ESTIMATION

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Riboflavin, vitamin B₂, serves in its phosphorylated form as the prosthetic group for a number of tissue enzymes important in biological oxidations (1). The involved substrates include glucose, lactic acid, the α amino acids, and other compounds of biological interest (1, 2, 3). Riboflavin deficiency has been described in man (4) and it seems possible that further clinical study of this vitamin may reveal alterations in its metabolism which will increase our knowledge of the role played by these enzymatic oxidation systems in health and in disease. To aid in interpreting such a study, a convenient method has been sought for following the urinary excretion of riboflavin.

It is known that riboflavin occurs in urine in the form of uroflavin, a pigment almost identical with it in composition¹ properties and vitamin activity (5) but the methods usually recommended for its determination (5, 6, 7, 8, 9) were not found by the writer to be sufficiently convenient or accurate for routine use. More specific and accurate determinations are possible, and smaller and more convenient volumes of urine (1 to 10 cc. instead of 100 to 500 cc.) can be used when the pigment is estimated not by its optical density but by its intense greenish yellow fluorescence². In order to do this, the effect of interfering substances must be avoided. With urines of normal individuals containing 0.5 gamma or more flavin per cc. this is readily accomplished by diluting down the salts and other materials to the point where they no longer affect fluorescence (10) and by destroying the greater part of other pig-

ments and fluorescent materials in a brief permanganate oxidation (5). With urines of certain patients containing less than 0.5 gamma per cc. some preliminary concentration of the uroflavin is necessary and can be much more conveniently and effectively carried out by using an adsorption column than by adding large quantities of adsorbing agent directly to urine (11, 12). In measuring the fluorescence of the column eluates or the diluted urine samples the usual ultra violet radiation is not satisfactory since it excites the fluorescence of too many substances. Measurements of flavin fluorescence are much more specific and much more accurate when carried out with an exciting beam of visual wavelength Mercury line at 4358A° and when the fluorometric photoelectric cell is protected by a yellow filter³. With such an arrangement the relationship between fluorescence and riboflavin concentration is linear for column eluates and diluted urine samples (Figures 1 and 2) particularly when the effect of any occasional alteration in optical density is corrected by means of the empirical curve illustrated in Figure 3.

METHODS

I Adsorption column procedure for measuring the uroflavin content of urines in general

An adsorption column⁴ 1 cm. in diameter and 15 cm. in length is set up with granular floridin earth⁵ which has been washed free of dust and

¹ The carbon analysis of the purest preparation isolated did not quite agree with that for riboflavin (Koschare (5)).

² Pfaltz & Bauer New York City. Model A is a suitable fluorometer provided with an additional cell for the measurement of optical density.

³ This optical arrangement is the work of Dr. D. J. Hennessey of Fordham University Chemistry Department and is to be described in detail elsewhere by him.

⁴ E. Machlett & Sons New York City. Usually a large number of columns and determinations are run simultaneously.

⁵ Pomeroy & Fischer New York City (30 mesh.) Recent preliminary experiments have indicated that a heat activated floridin and a synthetic material super

adsorbed gases by treatment with 2 per cent acetic acid and repeated rinsing with water. One to 10 cc of filtered urine⁶ at pH 5 are passed over the column. The total volume of the urine sample should be made up to about 10 cc so that the time of its passage will be about 5 minutes. The column is then washed several times with distilled water to remove the unadsorbed urinary sub-

gamma per cc aqueous solution of riboflavin⁷ fluoresces with an intensity of 20 galvanometer units while transmitting 50 galvanometer units of light. With this setting of the apparatus the column elution blank is fluorescence 6, transmission 49. Riboflavin added to this blank solution to give a concentration of 0.1 gamma per cc has a reading fluorescence 20, transmission 49. As

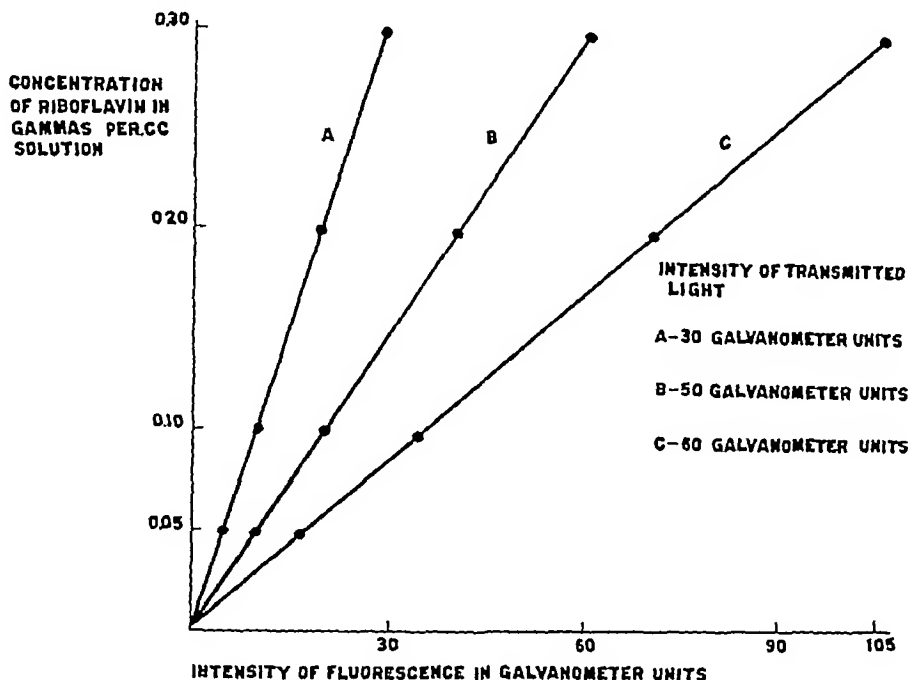


FIG 1 RELATIONSHIP OF FLUORESCENCE TO RIBOFLAVIN CONCENTRATION AT SEVERAL DIFFERENT INTENSITIES OF ILLUMINATION

stances and the uroflavin is eluted by passing over the column 20 cc of an aqueous solution of 20 per cent pyridine in 2 per cent acetic acid. This eluate is collected and cleared of oxidizable contaminants (5) by the addition of 2 drops of glacial acetic acid and 1 or 2 drops of 4 per cent potassium permanganate. The oxidation is stopped after 2 or 3 minutes by the addition of sufficient 3 per cent hydrogen peroxide to decolorize the solution (usually 1 to 5 drops), the total volume is made up to 25 cc with water, and the solution is filtered if necessary.

The fluorometer iris is set so that a standard 0.1

sorb, are superior to crude floridin earth. These materials were obtained from Mr O Fitzsimons, Floridin Co, Warren, Pa.

⁶ Twenty-four hour specimens are collected in dark bottles provided with 3 cc. glacial acetic acid and kept on ice during the period of collection.

the fluorescence of flavin in these pyridine solutions is not very stable, they should be protected from light and in taking readings a constant routine should be adopted.

The fluorescence and transmission of the eluates are measured and the uroflavin concentration in gammas per cc urine is calculated

$$\frac{\text{Fluorescence of unknown}^8 - 6}{20 - 6} \times 0.1 \times \frac{25}{\text{cc of urine used}}$$

equals uroflavin in gammas per cc urine

⁷ Merck & Co, Rahway, N J. This standard is made up fresh each day from a stock solution of 50 gammas per cc. The stock solution is stable for long periods when preserved with a little acetone and protected from light.

⁸ Corrected, if necessary, Figure 3 to compensate for any increase in optical density.

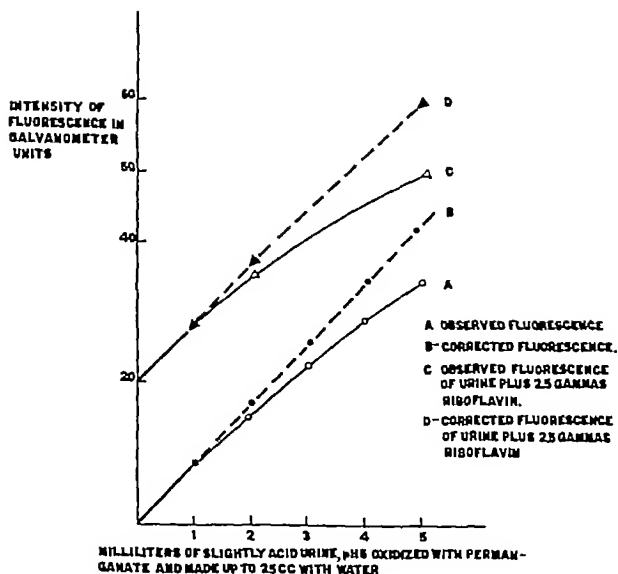


FIG. 2. MEASUREMENTS OF FLUORESCENCE IN SOLUTIONS OF DILUTED URINE, WITH AND WITHOUT CORRECTION FOR OPTICAL DENSITY OF SOLUTIONS

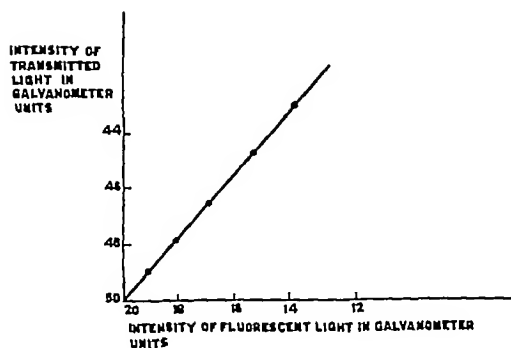


FIG. 3. RELATIONSHIP BETWEEN INTENSITY OF TRANSMITTED LIGHT AND INTENSITY OF FLUORESCENCE FOR A STANDARD RIBOFLAVIN SOLUTION OF 0.1 GAMMA PER CC.

TABLE I

The reliability of flavin determinations using the adsorption column technique

A Recovery of riboflavin from 10 cc aqueous solutions of standard riboflavin

Riboflavin in 10 cc standard gammas	Amount recovered in pyridine eluate per cent
1 25	107
2 5	100,100,100
5 0	98,100
7 5	99

B Duplicate determinations of uroflavin

Date	Subject	Uroflavin per cc of urine gammas	Amount urine used cubic centimeters
March 25	F	1 1 1 1 1 2	
April 22	F	1 1 1 1	5 10
May 6	F	2 0 1 7	
April 6	McD	0 25 0 25	
May 6	D	0 45 0 43	5 10
May 7	D	0 33 0 33	5 10
May 9	D	0 53 0 56	5 10
May 12	D	0 53 ± 05 (4 deter- minations)	

C Recovery of riboflavin added to urine

Uroflavin in specimen of urine gammas	Riboflavin added gammas	Recovery of added riboflavin per cent
2 3	2 5	87
0 63	2 5	100
1 6	2 2	87
2 7	2 5	107
5 6	2 5	104
3 0	2 5	106
1 8	2 5	85
5 4	2 5	100
17 0	2 5	96
16 0	2 5	100

Table I summarizes a study of the reliability of the method. Recovery of riboflavin from aqueous solutions of standard is good over a considerable range (section *A*). Duplicate determinations of uroflavin (section *B*) indicate a high degree of reproducibility which is not affected by altering the amount of urine used. This latter point is important because it demonstrates the method's independence of considerable variation

in the concentrations of organic and inorganic matter present during the adsorptions. Column filtrates and washings do not contain significant flavin* and a second eluate with 20 cc of pyridine reagent gives a fluorescence reading equal to that of the column blank. The recovery of riboflavin added to urine (section *C*) is as good as can be expected of an adsorption-elution procedure carried out in the presence of impurities.

II Direct method for estimating the uroflavin content of urines of high or moderate flavin concentration (0.5 gamma or more per cc)

One to 3 cc of filtered urine at pH 5 are diluted to 25 cc. with water, the urinary pigments are destroyed by oxidation with permanganate in the manner described for the pyridine eluates, and the fluorescence of the solution is measured directly in the fluorometer. The value of the blank is given by the reading taken after reduction of the flavin to its non-fluorescent leuco form.

TABLE II

Control conversions of riboflavin to lumiflavin

Amount of riboflavin in original solution gammas	Amount recovered as lumiflavin per cent
2 5	44
1 25	49
1 25	53
1 25	48

by the addition of a few particles of sodium hydrosulfite (5). When more than 2 or 3 cc of urine are used in the dilutions, considerable permanganate, 5 to 10 drops, may be needed for its oxidation and, with highly pigmented urines of low flavin content, this direct method is probably not very accurate (urine M table III). Whether it will prove to be sufficient for routine clinical use is not at present known. The agreement between these direct measurements and the values obtained by the floridin column method, Table III, is evidence for the completeness of the column procedure and indicates the combined effectiveness of the instrument's filter system and the permanganate oxidation in preventing other urinary substances from interfering with the determination of uroflavin fluorescence.

* The part played here by aquoflavin (Koscharka) is not clear to the writer.

As thus far discussed the uroflavin method has relied for its specificity upon the assumption that no material in urine other than uroflavin is capable of floridin earth adsorption, of elution by aqueous solutions of pyridine and acetic acid, resistance to permanganate oxidation in acid solution and greenish yellow fluorescence when illuminated by blue light. The method may be checked by determining uroflavin in the form of lumi uroflavin (5). As in the case of riboflavin (13, 14) this chloroform soluble pigment with intense green fluorescence can be formed from uroflavin by photolysis of strongly alkaline solutions in the cold. Unfortunately the procedure is at present of little practical use because of the difficulty experienced in obtaining quantitatively consistent conversions and the resulting necessity of control determinations (15, 16).

TABLE III

Agreement of uroflavin determinations by different techniques

Patient	Direct measurement of fluorescence of diluted urines treated with acid permanganate		Measurement by adsorption column technique		Measurement by lumiflavin technique	
	Uroflavin per cc. urine	Recovery of 2.5 gamma of added riboflavin	Riboflavin per cc. urine	Recovery of 2.5 gamma of added riboflavin	Riboflavin per cc. urine	Recovery of 2.5 gamma of added riboflavin
	gamma	per cent	gamma	per cent	gamma	per cent
McK	0.25		0.22		0.27	
M*	0.37	100	0.18	85	0.28	96
Wh	0.40	100	0.30	106	0.35	
S	0.70	100	0.54	100		
Fx	1.6		1.6	100	1.8	90
F	2.2	100	1.7	96	2.1	

* Concentrated, highly pigmented urine

† Recovery in this particular case means that added riboflavin produces the expected increment in fluorescence

III Method for determining uroflavin as lumi uroflavin

A preliminary alkalization of 5 cc. of urine is carried out in the dark and followed by acidification and extraction with chloroform so that the final chloroform extracts will contain only materials that have been rendered chloroform soluble by exposure to light. The extracted urine is brought into the light made up to half normal with concentrated sodium hydroxide and exposed in an ice bath to a 150 Watt lamp for 1½ to 2 hours. The pH is then brought to 3 with strong

acid and the lumiflavin is extracted from an aliquot of the urine by shaking with 20 cc. of chloroform. Control and recovery tubes are treated in a similar manner.

No data could be found in the literature relating the gram molecular fluorescence of riboflavin in water and the gram molecular fluorescence of lumiflavin in chloroform. This relationship was calculated by measuring the concentrations of the 2 pigments with a Koenig Martens spectrophotometer at 4700Å^o (17), and comparing their fluorescence in the Pfaltz Bauer fluorometer. For equal molecular numbers, lumiflavin in chloroform was found to have a fluorescence 2.25 times as great as that of riboflavin in water. It should be noted that this relationship may apply only to measurements made by using the filter system described with this fluorometer. Calculating from this factor of 2.25 and the results of the control and recovery experiments Tables II and III one finds that the determinations of uroflavin by this method are in reasonable agreement with those of the other two methods.

In determining the 24-hour uroflavin excretion of various patients some of whom may excrete very little flavin Table IV it is preferable to use the adsorption column procedure in general and to restrict the use of the direct method to normal urines or to urines containing large amounts of flavin such as those obtained during riboflavin tolerance tests. The lumiflavin method with its necessary controls is at present too time-consuming for routine use.

From the experiences of Table IV it can be concluded that there is a fairly general dependence of uroflavin excretion upon riboflavin intake (18). This is particularly well illustrated by the patients D, McK and Fx and an examination of the actual dietary intakes of the other patients and subjects confirmed this impression, for example patients G and W though on regular diets were both sick and not eating well. Patient Kz on a high carbohydrate high vitamin diet was jaundiced and sick. The normal subjects F and Km excreted considerably more uroflavin when liver or synthetic riboflavin was added to their diets. Normal rats on a riboflavin-deficient diet excreted very little uroflavin at a time when the riboflavin content of their livers had fallen to 40 per cent of normal. When placed on a complete diet their

TABLE IV

*Twenty-four hour excretion of uroflavin**A Normal subjects eating their usual diets*

Subject	Uroflavin excretion <i>gamma</i>
C	850
G	1100
H	700
F	1700
K	1400

B Normal subjects on regular hospital diets of 2400 calories

Subject	Uroflavin excretion Daily determinations for 5 days <i>gamma</i>
F	1200 ± 200
Km	1000 ± 100

C Patients on various hospital diets

Subject	Type of diet	Uroflavin excretion <i>gamma</i>
G	Regular	450
W	Regular	430
Ch	Regular	560
Kz	High carbohydrate, high vitamin	450
McD	Low potassium	170
McD	Low potassium	220
D	Low potassium	260
D	Low potassium	200
D	Low potassium	250
D	Regular, eating heavily	1200
McK	Low residue, high caloric	200
McK	Low residue, high caloric, plus 1 mgm Riboflavin	500
McK	Low residue, high caloric, plus 2 mgm Riboflavin	800
Fx	1 liter 10 per cent cream every day for one week *	1300

* Contained 1.5 milligrams lactoflavin by direct measurement of acetone filtrate

uroflavin excretion increased ten-fold. No observations were made of the bearing which phosphorylation (19, 20) may have upon riboflavin utilization and uroflavin excretion, but some evaluation of this factor and that of intestinal adsorption is being attempted by means of flavin tolerance studies now in progress.

SUMMARY

The importance of studying the metabolism of riboflavin has been pointed out.

Fluorometric methods for estimating the urinary excretion of riboflavin have been described.

Studies have been presented to show that these methods are convenient and accurate enough for clinical use.

The relationship of riboflavin intake to uroflavin excretion has been discussed.

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SULFAPYRIDINE IN EXPERIMENTAL PNEUMOCOCCIC PNEUMONIA IN THE DOG¹

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Previously reported studies of the action of sulfapyridine in experimental pneumococcic infection have been carried out in the mouse rabbit and rat—species which, unlike man are highly susceptible to the pneumococcus

Whitby (1) found that mice infected with Type I pneumococci and treated with sulfapyridine possessed after recovery considerable immunity to the same organism. A similar observation was made by Schmidt and Hilles (2). Whether acquired immunity plays an essential rôle in the mechanism of recovery in treated mice is not known, but there is some indirect evidence to suggest that it may do so. Especially significant is MacLeod's (3) observation on mice infected with pneumococci Types I II and III, respectively that the survival rate for each type was closely similar in actively immunized and in sulfapyridine treated groups. Furthermore there is clearly demonstrable a synergistic antipneumococcic action *in vivo* between artificially induced immunity and sulfapyridine as shown by the work of Powell and Jameson (4) MacLean Rogers and Fleming (5 6) and Kepl and Gunn (7). From these considerations it would seem that natural antipneumococcic resistance of a relatively high order such as that possessed by human beings as a group (8) may enhance the effectiveness of sulfapyridine.

The dog resembles man in ability to localize pneumococci in the tissues and in degree of natural humoral immunity. O. H. Robertson and co-workers (9 10) have shown that lobar pneumonia, which in all essential respects is comparable to the human disease can be produced in the dog. They have demonstrated further that mortality in the experimental disease can be regulated within certain limits by varying the infect-

ing dosage of pneumococci that there is a direct relationship between extent of pulmonary involvement and death rate, and that there is a steadily rising mortality with increasing degrees of leukopenia and of bacteremia which is of definite prognostic significance even at 24 hours (11).

The present report concerns observations on the course of pneumococcus Type I pneumonia in 34 dogs treated with sulfapyridine² the administration of which was begun before infection and at various stages of the disease. An infecting dose of culture was selected which would result in a disease severe enough to provide a crucial test for the drug but not so fulminating that spontaneous recovery would be impossible. Simultaneous untreated controls were employed throughout the study.

Canine pneumonia differs from the human disease in having a more rapid course. This feature is to be borne in mind when considering the time at which treatment was begun in this investigation.

METHODS AND MATERIALS

Dogs weighing 8 to 15 kilograms were infected intrabronchially by the method of Robertson and Fox (11) with 1 cc. of a 16-hour pneumococcus culture followed by 3 cc. of animal mucus. The microorganisms were separated from the culture medium and resuspended in 1 cc. of Locke's solution containing 0.1 per cent gelatin. In the special instances where 0.02 cc. of culture was used, it was mixed directly with 1 cc. of a 6 per cent starch-broth medium no mucus being employed. The same strain of Type I pneumococcus (A₁) was used throughout. It had been passed through a rabbit every 6 weeks, and through a dog on 2 occasions, to maintain its virulence.

Sulfapyridine was administered orally in capsules or compressed tablets.

The concentration of sulfapyridine in the blood and exudates was determined by the method of Marshall and

¹ Preliminary report appeared in the Proceedings of the Society for Experimental Biology and Medicine, 1939 41, 459-462.

² The sulfapyridine used in this investigation was kindly furnished by Merck and Co.

Litchfield³ (12) The pneumococcal-promoting power of serum was tested in serum-leukocyte mixtures by the method of Robertson and Sia (13) When color change appeared, indicating growth, the tubes were opened and their contents examined for organisms in stained films All tubes showing no color change were examined in the same way at 72 hours The test is so assembled that 0.3 cc of serum is diluted with the other constituents to a final volume of 0.5 cc. Blood cultures were made in the usual manner, using 1 cc. of blood to 10 cc. of melted agar In addition, after treatment had been instituted, cultures were also made with 1 cc. of blood in 25 cc. of broth to achieve even greater dilution of drug present in the blood.

Roentgenograms of the chest (14) were made at the time treatment was begun and almost daily thereafter until recovery or death occurred. Body temperatures were taken rectally, at least twice a day, morning and late afternoon. Pulmonary exudate for bacteriologic examination was obtained postmortem by piercing the seared surface of the lung with a sterile capillary pipette.

EXPERIMENTAL

Outcome and duration of the disease Effect of treatment when begun within 24 hours of infection

In the 32 dogs employed as controls during this study, the mortality was 50 per cent, and the disease was brief in duration whatever the outcome (Table I) Within 72 hours of the time of infection the temperature had fallen to normal in all but 2 of the 16 animals that ultimately survived Of the 16 that died, only 3 lived for more than 96 hours

Twenty-four dogs were given sulfapyridine on the first day of the disease, beginning 3 to 24 hours after infection, and 3 times daily thereafter for 2 to 5 days (Table I) The total quantity of drug that each received was 0.25 to 0.8 gram per kilo of body weight, an average of 0.5 gram per kilo In 9 instances where serial blood determinations were made, the content of free sulfapyridine was generally 2 to 8 mgm per cent during the first 2 days of administration, having reached that level within 6 hours (Table III)

Of the 24 dogs so treated, none died Eleven were used in other experiments 10 to 60 days after recovery, when x-ray examination showed

³ The figures in our data represent only the free sulfapyridine concentrations in the blood and empyemic fluid

TABLE I

Mortality and duration of fever in controls and in dogs treated with sulfapyridine

Treatment begun within 24 hours after infection

Sulfapyridine		Number of dogs	Duration of fever * Number of dogs showing drop of temperature to normal					Total mortality
Begun at intervals after infection	Total amount given		within 24 hours	between 24 and 48 hours	between 48 and 72 hours	between 72 and 96 hours	after 96 hours	
hours	grams							per cent
3	5 to 7	10	0	5	3	1	1	0
12	5 to 6	5	0	3	2			
18 to 24	3 to 6	9	1	2	5	1		
Control dogs								50
Surviving		16	1	4	9	1	1	
Dying†		16	1	4	4	4	3	

Dosage of drug initial dose 2 or 3 grams, followed in 6 to 8 hours with $\frac{1}{2}$ gram three times a day for 2 to 5 days

* Temperatures (rectal) above 102.9° F considered to indicate fever

† Figures in columns 4 to 8 indicate time of death

beginning or complete resolution of the pneumonia The others were sacrificed for pathological study 6 to 40 days after infection, or killed because they had developed "snuffles" several days or weeks after having recovered from the experimental pneumococcal disease Cultures taken postmortem from lesions not yet resolved yielded no pneumococci The lungs of 3 dogs with "snuffles" contained gram-positive cocci and gram-negative bacilli⁴

The duration of fever (Table I) in animals receiving the first dose of sulfapyridine on the 18th or 24th hour of the disease was practically the same as it was in the controls that survived When treatment was begun as early as the 12th hour, more than one-half of the dogs showed a normal temperature by the 48th hour, whereas less than one-third of the surviving controls did so

Nearly all of the treated dogs continued to have fever for at least 24 hours after the beginning of treatment

⁴ In this laboratory these organisms have often been found in the lungs of dogs with acute epidemic respiratory disease commonly called "snuffles"

Relation to outcome of extent of pulmonary involvement, white blood count and occurrence of bacteremia at the 24th hour of the disease

In the control dogs (Table II) the close correlation between extent of lung involvement and outcome is evident. When the pulmonary lesion at 24 hours involved not more than one-sixth of the total lung field, as judged from an x-ray film (approximately one lobe), all but one of 9 dogs recovered, when it occupied one-half or more of the total lung field 9 of 13 died. The presence

Effect of early treatment on extent of pulmonary lesion and on incidence of leukopenia and bacteremia at the 24th hour of the disease

The pulmonary lesion at 24 hours (Table II) involved one half or more of the total lung field in nearly one-half of the control dogs. Such an extensive pneumonia occurred in only one-fifth of those treated within 12 hours of inoculation. However, there was involvement of at least one-third of the lung field at the 24th hour in 9 out of the 10 dogs treated as early as the 3rd hour.

Extension of the pneumonic process after 24 hours was seen by x-ray in only one of the controls that survived. Of those that died one half showed a gross spread, as determined from a comparison of the size of the x-ray shadow occurring at the 24th hour and the extent of consolidation found at autopsy.

Among the treated dogs spread occurred after the 24th hour of the disease in only 2 of the 15 treated within 12 hours, in one instance between the 24th and 48th hour and in the other between the 48th and 72nd hour as well. It occurred in 5 of the 9 dogs not treated until the 18th or 24th hour but in only one of these did it continue beyond the 48th hour.

Bacteremia at 24 hours (Table II) was found in more than one-third of the control dogs but in only 2 of the 15 treated within 12 hours of infection and in the latter it was minimal and disappeared by the 48th hour. Early treatment did not appreciably reduce the incidence of extreme leukopenia (Table II).

Effect of treatment on antipneumococcal activity of the blood

The sera of 11 of the 24 recovered dogs in the treated series were tested for mouse protective action 3 to 22 days after recovery and this property was found in the sera of 8 of the animals.

Serum was collected before infection and during the course of treatment from dogs receiving the first dose of sulfapyridine 18 or 24 hours after infection and examined for pneumococcal-promoting power in serum leukocyte mixtures by the method of Robertson and Sia. The results of these tests revealed in general a slight enhancement of this property within 24 hours.

TABLE II

Extent of pulmonary involvement, white blood count and incidence of bacteremia 24 hours after infection in controls and in dogs treated with sulfapyridine. Relationship to outcome

Treatment begun within 24 hours after infection

Treatment begun at intervals after infection	Total number of dogs	Extent of pulmonary lesion (x-ray)			White blood count		Incidence of bacteremia			
		Number of dogs with involvement of			Number of dogs with white blood count of		Number of dogs with colony count per cc. of blood			
		1/6 or less of total lung field	1/3 to 1/2 of total lung field	1/2 or more of total lung field	2,000 and above	1,000 and below	0	1 to 20	21 to 100	101 or more
<i>Sera</i>										
3	10	1	6	3	7	3	8	2	0	0
13	5	4	1	0	3	2†	8	0†	0	0
18-24	9	2	4	3	6	3	8	1	1	1
<i>Controls</i>	31 (13)	9 (1)	9 (3)	13 (9)	23 (9)	9 (6)	20 (7)	6 (3)	1 (1)	4 (4)

Figures in parentheses indicate number of dogs dying.

* One dog died in less than 24 hours.

† Present at 12 hours.

‡ Positive in 2 at 12 hours.

of bacteremia 24 hours after infection was associated with a fatal outcome in 8 of the 11 control dogs in which it occurred. None survived whose blood at 24 hours contained on culture more than 20 colonies per cc. The relationship between white blood count and outcome is less obvious in this small series than in the much larger one reported by Robertson and Fox (11).

In the group receiving the first dose of sulfapyridine 18 or 24 hours after inoculation (Table II) there was an expected incidence of widespread lung involvement, blood stream invasion and profound leukopenia, yet all recovered.

TABLE III

Course of bacteremia, pneumococcal-promoting power of the serum, and concentration of sulfapyridine in the blood

Dogs that received the first dose of sulfapyridine 18 or 24 hours after infection

Dog number	Time of observations	Blood culture	Pneumo-coccal-promoting power of serum	Concentration of sulfapyridine in the blood
		colonies pneumococci per cc. blood	number of pneumococci killed	mgm. per cent
220T	Before infection			
	At time treatment was begun	0	10^3 10^5	
	Intervals after 6 hours	0	10^4	3.5
	beginning of 24 hours	0	10^4	5.7
	treatment 48 hours	—	—	2.4
225T	Before infection			
	At time treatment was begun	0	10^3	
	Intervals after 6 hours	0	10^3	3.4
	beginning of 24 hours	0	—	6.4
	treatment 48 hours	0	10^3	4.5
238T	Before infection		10^4 10^5	
	At time treatment was begun	0	10^4	
	Intervals after 6 hours	0	—	4.2
	beginning of 24 hours	0	10^4	6.4
	treatment 48 hours	—	—	1.4
241T	Before infection			
	At time treatment was begun	0	—	
	Intervals after 6 hours	0	—	6.0
	beginning of 24 hours	0	—	1.6
	treatment 48 hours	—	—	1.4
232T	Before infection		10^3 10^5	
	At time treatment was begun	0	—	
	Intervals after 12 hours	0	10^4	6.0
	beginning of 24 hours	0	—	5.2
	treatment 48 hours	0	—	1.6
249T	Before infection		10^3	
	At time treatment was begun	0	10^4	
	Intervals after 24 hours	0	10^4	3.1
	treatment 48 hours	0	—	3.6
219T	Before infection		10^4 10^5	
	At time treatment was begun	230	10^4	
	Intervals after 6 hours	39	10^4	1.6
	beginning of 12 hours	10	—	5.6
	treatment 24 hours	pos.*	10^4	4.8
	48 hours	0†	—	3.6
222T	Before infection		10^3 10^4	
	At time treatment was begun	1	10^4	
	Intervals after 6 hours	0	10^4	5.0
	beginning of 24 hours	0	—	4.6
	treatment 48 hours	0	—	trace
229T	Before infection		10^4 10^5	
	At time treatment was begun	26	10^4	
	Intervals after 12 hours	pos.*	10^4	11.0
	beginning of 24 hours	0	10^4	8.4
	treatment 48 hours	0	—	1.1

* = Growth in broth culture only

† = Blood cultures made at 72nd and 96th hour also negative

— = Not done

Effect of treatment on the course of bacteremia

Three of the dogs receiving the first dose of sulfapyridine 18 or 24 hours after infection were bacteremic at the time treatment was begun (Table III). The colony counts were respectively 1, 26 and 230 per cc of blood. Blood cultures made 24 hours later were negative in poured agar plates, but in one instance still positive in the broth. In only 1 of the 3 animals had there been a measurable decrease in the titer of circulating antibody by the time treatment was started, and the decrease was very slight. During treatment the serum of this animal exhibited pneumococcal-promoting power greater than it had possessed before infection.

To test the effectiveness of sulfapyridine in the presence of more massive pneumococcemia, 4 additional bacteremic dogs were selected for treatment, all but 1 having a colony count of more than 1,000 per cc of blood. Each was given a total of 57 or 6 grams of the drug. Its administration was begun with a large dose (2 or 3 grams) 30 to 72 hours after infection, and continued with 1 gram a day in divided doses until death or apparent recovery from infection occurred. All died ultimately.

One animal (Table IV, 284 T) in the 30th hour of the disease, the time at which treatment was instituted, had a bacteremia of 10,000 colonies per cc of blood, and its serum had no demonstrable pneumococcal-promoting action. Blood drawn 18 hours later (on the 3rd day of the disease) was sterile on culture, and the serum exhibited in a serum-leukocyte mixture pneumococcal action against 1,000 pneumococci. A serum sample obtained on the 4th day when the blood contained 4 mgm per cent of sulfapyridine showed pneumococcal-promoting properties to an even greater degree, but did not prevent the growth of an inoculum of 10 organisms after the serum had been subjected to a temperature of 56° C for one-half hour. Moreover, the sample contained no demonstrable mouse-protective antibodies characteristic of acquired immunity. Death occurred on the 5th day. At autopsy the pericardial sac was found distended with about 50 cc of thick purulent exudate which on microscopic examination contained innumerable diplococci. When the Neufeld test was applied to

initiation of treatment (Table III). When the samples possessing this increased activity were tested in the same way, but with hemoglobin solution substituted for the leukocyte suspension, there was growth of even the smallest inoculum of pneumococci (approximately 10 organisms)

TABLE IV

Course of disease in 2 of 4 bacteremic dogs in which treatment was begun later than 24 hours after infection

Day of disease	Dog No. 284 T							Dog No. 288 T							
	Before infection	2nd			3rd	4th	5th	Before infection	2nd	3rd	4th	5th	6th	7th	
		24th hour	30th hour	36th hour											
Dosage of sulfapyridine grams			2	1	1	1	1				2	7	1	1	1*
Blood culture colonies pneumococci per cc. of blood		2 000	10 000	1 500	0	11	1		9	1 800	1 100	2	1	1	0†
Pneumococcal promoting power of serum number of pneumococci killed in serum-leukocyte mixture			0		10 ²	10 ⁴ †		10 ⁴ 10 ²		0				10 ⁴	
Concentration of sulfapyridine in blood mgm per cent				4.7	10.4	4.0	2.1						10.0	4.0	
White blood count thousands	6.5	1.6	1.8	1.8	2.2	4.8	8.2	9.6	3.3	4	8	16	12	24	
Extent of pulmonary involvement (x ray)			LLL LML									LLL RUL			

Outcome 284 T died on 5th day Purulent pericarditis
 288 T died on 11th day Anemia and jaundice.

* Final dose of drug on 7th day

† No mouse protective action

‡ Blood culture on 8th and 9th days also sterile.

them all the visible organisms showed a typical capsular swelling. The exudate contained 5 mgm per cent of sulfapyridine. No pneumococci could be cultured from the lungs or heart's blood.

An x ray of the chest taken on the 2nd day of the disease a few hours before treatment was begun showed a cardiac shadow not unusual in size and shape. On the 3rd day the silhouette was slightly larger than before and on the 5th day it was globular and much enlarged. The red blood count on the 5th day was 6 100 000.

Another animal (not shown in the table) pursued a similar course. Treatment was begun on the 5th day when the blood on culture contained 6 000 colonies per cc. and the serum no longer possessed pneumococcal promoting action. By the 7th day the colony count had fallen to 50 but on the 8th day the day of death it rose to 10 000. Empyema was found at autopsy. The exudate contained Type I pneumococci and a concentration of sulfapyridine of 0.9 mgm per cent. It was not possible to ascertain when the empyema had begun. However there was a suggestive shadow already present on the day treatment was started. The red blood count on the 5th day was 5 300 000.

Among the 16 control dogs that died, 6 of the 7 that had lived for more than 72 hours were found at autopsy to have empyema. This complication did not occur in those dying earlier in the disease. Purulent pericarditis was not observed in the control dogs.

In the other 2 members of the group of 4 bacteremic dogs specially selected for treatment, severe anemia and jaundice developed. In each case the blood became sterile several days before death and autopsy cultures from lungs and heart's blood yielded no pneumococci. One of the dogs (Table IV 288 T) was treated on the 4th day of the disease when its blood on culture contained 1 100 colonies per cc. The pneumococcal promoting power of the serum had already disappeared. By the 6th day the colony count had dropped to one and anti pneumococcal action of the serum had returned. Blood collected on the 9th day contained antibodies characteristic of acquired immunity; the 6th day's sample was not tested for heat stable immune bodies. The temperature had become normal on the 5th day and the dog seemed to be recovering. The last dose of drug was given on the 8th day. Death occurred 3 days later. At autopsy the

and mucous membranes were deeply icteric. Unclotted blood from the heart contained 1,100,000 red cells per cubic millimeter, and they showed striking variation in size, shape, and staining qualities

The fourth dog was first treated on the 4th day of the disease, when there was a bacteremia of 78 colonies per cc of blood. The serum had retained some pneumococcal-promoting activity, but possessed much less than it did before infection. The blood became sterile on the 5th day. The serum on that day had no mouse-protective action. The temperature became normal on the 5th day, treatment was discontinued on the 6th day. The red blood count on the 7th day was 3,400,000. The dog died on the 8th day, presenting the same findings as the preceding animal.

Production of lobar pneumonia in dogs receiving sulfapyridine prior to infection

In 6 dogs (Table V) a fully developed lobar pneumonia evolved in 24 hours despite the administration of large doses of sulfapyridine before and after inoculation, and despite its presence in the blood in concentrations equal to or greater than those obtaining in experiments where the drug was regularly curative.

The dogs were sacrificed 24 hours after infection. Four were definitely ill at that time, having temperatures of 103° or more. None were leukopenic or bacteremic, however. At autopsy there was, with one exception, consolidation of at least the greater part of the lobe which received the infecting inoculum, and in 3 instances there were small lesions in other lobes as well. In the exudate from 4 of the primary lesions numerous pneumococci were readily seen. They showed no alteration in morphology or staining qualities as observed by the Gram stain. When the exudate was mixed with Type I antipneumococcal rabbit serum the organism showed immediately a positive "quellung" reaction. There appeared to be no alteration of the capsules. Cultures from these lesions and from one other contained type-specific pneumococci.

DISCUSSION

The potential efficacy of sulfapyridine as a life-saving therapeutic agent in pneumococcal infec-

TABLE V
Production of lobar pneumonia in dogs receiving sulfapyridine prior to infection
Dogs sacrificed 24 hours after infection

Dog number	Infecting dose of culture	Concentration of drug in the blood		Extent of lung involvement at autopsy	Lung puncture at autopsy			Rectal temperature when sacrificed
		When infected	When sacrificed		Culture	Direct examination		
						Gram-positive diplococci	Newfeld reaction	
	cc.	mgn. per cent	mgn. per cent	lobes				degrees Fahrenheit
251T	1.0	10.0	2.8	1	Pneumococcus	+	+	104.0
281T	1.0	4.8	3.7	1	Pneumococcus	+	+	104.0
271T	0.02*	3.3	3.6	1/3	Pneumococcus	0	-	103.8
273T	0.03*	18.0	2.8	1	0	0	-	102.5
273T	0.02*	12.0	5.1	<1	Pneumococcus	+	+	103.3
280T	0.02*	12.8	5.0	>1	Pneumococcus	+	+	10° 4

* Suspended in 1 cc of starch broth medium

> = More than

< = Less than

Total quantity of drug before infection: 271 T, 272 T = 3 grams, 273 T, 280 T, 281 T = 4 grams, 251 T = 5 grams, given in 2 divided doses, the first dose 18 to 24 hours before infection, the second 6 to 12 hours before infection.

Total quantity of drug after infection: 271 T, 272 T, 251 T = 2 grams, 273 T, 280 T, 281 T = 4 grams, given in 2 divided doses, the first dose 6 hours after infection, the second 12 to 18 hours after infection.

tion is especially evident in the recovery of certain of the dogs showing a well advanced disease by the end of 18 to 24 hours, when treatment was begun. In several of these instances the findings were such as to indicate a high probability of fatal termination. That it is able to promote an arrest of infection is also shown by the brevity of the febrile stage, the restricted size of the pulmonary lesions, and the limited occurrence of bacteremia in dogs treated earlier in the disease. A pronounced antipneumococcal effect of the drug is obvious even in those instances where treatment was instituted at a time when the blood contained thousands of pneumococci per cc., though focal suppurative complications with toxemia of severe infection and drug intoxication prevented ultimate recovery.

A single strain of pneumococcus Type I was employed in these experiments. Because there is evidence that the several types of pneumococci (1, 15), and also strains of the same type (6), vary in their susceptibility to sulfapyridine, broad conclusions concerning its efficacy in the treatment of canine pneumonia are not permissible at this time. However, the results of the present study,

obtained in a highly fatal lobar pneumonia in an animal with antipneumococcal resistance comparable to that of man, are in complete accord with clinical observations (16) and indicate that the drug possesses remarkable curative properties.

The ability of sulfapyridine to re-enforce the body's natural antipneumococcal defense is reflected in the augmented pneumococcal promoting power of serum collected during the course of treatment as tested in serum leukocyte mixtures. This increased action may be attributable to an inhibition of the bacterial growth which occurs before completion of phagocytosis. An alternate explanation is that the drug renders pneumococci more susceptible to phagocytosis perhaps by causing alteration of the capsules.

Whitby (1) has described changes in the capsule of pneumococci obtained from the peritoneal exudate of mice treated with sulfapyridine and has stated that after 4 hours in large numbers of cocci no capsules could be found by staining methods. He has pointed out that this capsular degeneration was an *in vivo* phenomenon and that it may have been a secondary effect representing a process whereby the body rids itself of dead pneumococci. Telling and Oliver (17) found that after patients had been treated with sulfapyridine the organisms isolated from sputum had lost capsules and type-specificity and that these were restored by repeated mouse passage. Hilles and Schmidt (18), and Greey, MacLaren and Lucas (19) have reported isolation of non-encapsulated pneumococci from the blood of sulfapyridine-treated mice. Long (20) and Reid (21) have been unable to find specific capsular changes in the peritoneal exudates of mice. Fleming (5b) observed no changes in the capsules of pneumococci grown *in vitro* in the blood of patients in the presence of sulfapyridine.

We found normal capsular swelling regularly when the Neufeld test was applied to pneumococci growing in blood cultures made during the course of treatment and to those obtained directly from the lungs of dogs that received the drug before and after infection and were sacrificed 24 hours afterwards. If loss of capsule had occurred it is unlikely that non-encapsulated pneumococci could have existed except momentarily in the presence of actively phagocytizing leukocytes. Thus while our observations do not exclude the

possibility that sulfapyridine produces capsular changes, they provide no evidence for such an effect.

To account for the drug's curative action in canine pneumonia, no more than a bacteriostatic effect need be assumed, for if not overwhelmed by rapid pneumococcus growth the immediate defense mechanism of the body (22) is able to retard the progress of infection until the normal recovery processes (including the macrophage reaction in the lung (23)) can mobilize. That the natural antipneumococcal defense at least the humoral component can again manifest activity even after having been markedly depressed (24), is indicated by the reappearance of heat labile opsonins in the sera of dogs 284 T and 288 T as bacteremia diminished with treatment.

It follows then that any noteworthy reduction in rate of pneumococcus growth and the resultant slowing of outward flow of pneumococcus laden edema fluid from the lesion would retard spread of infection inasmuch as polymorphonuclear leukocytes at the spreading border are actively phagocytic, at least early in the course of the disease (22). That such a result is not immediate is clearly evident in those experiments in which administration of the drug prior to infection failed to prevent the development of the experimental lesion. In untreated dogs the smaller infecting dose employed (0.02 cc. of culture in 1 cc. of starch broth paste) has been found by Robertson and Fox (11) to produce a lesion which on the average occupies eventually but one-quarter of the lung field by x ray (approximately 1 and one-half lobes), and results in a mortality of only 8 per cent.

This evidence of the delayed action of sulfapyridine is comparable to the findings of McIntosh and Whitby (25) in experiments on mice and on citrated rabbit's blood.

That the normal defensive elements play an essential part in bringing about recovery in treated dogs is indicated by the persistence of purulent complications in dogs 275 T and 284 T. The findings in the latter dog are especially significant for while the blood and lungs became free of pneumococci as determined at autopsy the pericardial exudate contained myriads of pneumococci despite the presence of sulfapyridine in concentration of 5 mgm. per cent. It is known that

leukocytes in such exudates lose activity after a day or so

Mouse-protective antibodies, characteristic of acquired immunity, were found in dogs recovering with treatment in about the same frequency and titer as ordinarily found in dogs recovering spontaneously. None were found before the 3rd day after recovery in 4 dogs tested. Thus, here as well as in spontaneous recovery (8, 26), the role of these substances cannot be evaluated.

Whether or not drug intoxication was responsible for the profound anemia observed in 2 dogs cannot be determined. Powell and Chen (27) have given normal dogs as much as 1 gram of sulfapyridine per kilo of body weight for as long as 4 weeks without finding any significant reduction in red blood count. In this laboratory severe anemia and jaundice have been observed otherwise but once in over a thousand dogs with experimental pneumonia, and that was in an actively immunized animal that had recovered from a 5 days' illness. However, many dogs have recovered spontaneously from prolonged and severe infections without developing the disorder. The fact that in the present series the anemia occurred only in dogs with severe infection suggests a dual etiology, namely, drug intoxications and toxemia of infection.

SUMMARY

1 In the dog, a species with natural antipneumococcic resistance comparable to that of the human being, sulfapyridine exerted a marked curative effect in a highly fatal Type I pneumococcic pneumonia.

2 The pulmonary lesion seldom spread after the 24th hour of treatment but it was not immediately arrested. Administration of sulfapyridine before infection did not prevent the evolution of a fully developed lobar pneumonia.

3 Most of the animals had fever for at least 24 hours after the beginning of treatment.

4 Treatment inhibited blood-stream invasion. Bacteremia already established disappeared within 24 hours or the colony count fell sharply.

5 Influence of the drug was evident even in far advanced stages of the disease but its effectiveness was limited in the presence of suppurative complications.

6 During treatment the serum exhibited increased pneumococcidal-promoting action in serum-leukocyte mixtures or regained that property when it had disappeared.

7 Pneumococci obtained from lungs, blood, and pyemic exudate showed no alteration of the capsules.

8 Treatment was followed by hemolytic anemia in 2 instances.

9 The mechanism of the drug's therapeutic action is discussed.

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STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS I THE TOTAL PHOSPHORUS METABOLISM OF NORMAL AND LEUKEMIC MICE¹

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In a previous study (1) using 'tagged' phosphorus, it was found that lymphomatous mice handle a single dose of sodium phosphate in a manner different from normal animals. Lymphomatous tissue absorbed or exchanged phosphorus in greater degree than normal lymph nodes and this seemed to occur at the expense of the uptake in bone in these animals.

Before investigating this question further in leukemic animals, it seemed important first to determine the content of total phosphorus in various lymphomatous, leukemic and normal tissues. One might suspect that the greater exchange of phosphorus in lymphomatous tissue was related to a greater total phosphorus content. The analyses were carried out using the methods of Pregl (2) for total phosphorus. The animals were young adults of the Strong A strain (3), with males and females equally distributed between the normal and leukemic animals. Most of them were given the disease (4) by the intravenous injection of leukemic cells but a few were inoculated subcutaneously the latter method resulting in the appearance of a local lymphoma followed later by the generalized disease. In all instances the animals were killed by breaking the neck and the tissues were immediately weighed and prepared for phosphorus analysis. In all cases, gross evidence of generalized leukemia was found in the leukemic group. Examination of Table I reveals that there is no significant difference in the total phosphorus contents of leukemic spleens leukemic lymph nodes, and leukemic bones² when compared with the respective normal tissues, in spite of the fact that, in leukemic animals, all of these tissues are infiltrated with leukemic cells. Likewise, the values for skeletal muscle are the same

in the two groups of animals. This latter finding was to be expected, since there usually is no leukemic infiltration of skeletal muscle in this disease. Finally, tumor or lymphomatous tissue has a significantly lower total phosphorus content than its analogue—normal lymph node. Despite the fact that the phosphorus content of leukemic liver may be significantly lower than that of normal liver these results show that any difference in the exchange of a single dose of phosphorus observed in lymphomatous or leukemic animals cannot be explained on the basis of differences in the total phosphorus values.

We wish to report here further studies on the comparative uptake and turnover of "labelled" phosphorus in a large group of normal and leukemic animals.

PROCEDURE

In the experiments to be reported here, adult animals of the A strain were used, and the leukemia was transmitted to them by the intravenous inoculation of a suspension of lymphomatous cells. After the onset of the leukemia, as evidenced by enlarged spleens and lymph nodes, leukemic and control animals were given intraperitoneally a single tracer dose of radioactive phosphorus in the form of an isotonic solution of neutral sodium phosphate. At various periods thereafter leukemic and control animals were sacrificed and their tissues were freshly weighed, dried and ashed at 450° C. Afterwards the ashes were measured for beta ray activity using a Lauritsen type electroscope or an electrometer. The radioactive phosphorus (P^{32}) which has a half life of 14.3 days was prepared in the Berkeley cyclotron (5) by the bombardment of red phosphorus (P^0) with high speed deuterons. Thereafter it was converted to a neutral solution of sodium phosphate.

Forty female mice, varying in ages from 5 to 8 months were used in this study. The animals were divided into eight groups of 5 mice each, and 20 of them were given intravenously in 0.1 cc. 9,300,000 lymphomatous cells. Groups of 5 animals were placed in individual cages, having raised wire floors so that coprophagy was avoided. All animals were given free access to water and they were fed on dog chow. The weighed daily food in

¹ This work was made possible through a grant from the Josiah Macy Jr. Foundation.

² The whole femurs were used in these analyses.

TABLE I
Total phosphorus in tissues of normal and leukemic mice*

Tissue	Normal or leukemic	Number of animals	Mean mg P/g	σ_x	σ_M	$M_N - M_L$	$\sigma_{M_N - M_L}$	
Liver	N	46	3.86	0.35	0.051	0.27	0.074	Difference is of possible significance
Liver	L	39	3.59	0.33	0.053			
Spleen	N	22	4.58	0.52	0.11	0.19	0.12	Difference is not significant
Spleen	L	39	4.77	0.27	0.043			
Lymph node	N	40	5.67	0.74	0.117	0.14	0.265	Difference is not significant
Lymph node	L	27	5.81	1.24	0.238			
Bone	N	40	75.6	9.9	1.57	2.4	2.45	Difference is not significant
Bone	L	28	73.2	9.9	1.87			
Muscle	N	37	3.02	0.47	0.064	0.02	0.084	Difference is not significant
Muscle	L	53	3.00	0.33	0.054			
Tumor (lymphoma)		29	4.14	0.34	0.064			

* σ_x = Standard deviation of individual values = $\sqrt{\sum x^2/n}$ where x equals the difference of the individual values from the mean

$M_N - M_L$ = Difference of mean values for normal and leukemic tissues

σ_M = Standard error of the mean = σ_x/\sqrt{n}

$\sigma_{M_N - M_L}$ = Standard error of the difference of means = $\sqrt{\sigma_{M_N}^2 + \sigma_{M_L}^2}$

Criterion of significance. If the difference of the means exceeds three times the standard error of the difference of the means, the difference is considered to be of possible significance

take of each group of leukemic animals determined the amount of food given to its control group on the following day. This tended to make uniform the total daily phosphorus intake of the two groups. Each group of leukemic animals exhibited enlarged spleens when the phosphorus was administered, and all animals (leukemic and control) were sacrificed 16 days after inoculation with leukemia. The radio-phosphorus was administered intraperitoneally in the form of 0.5 cc. of a solution containing 7 mgm. sodium phosphate at a pH of 7, having a P^{32} activity of 95 microcuries when the first group was injected. Five normal and 5 leukemic animals were each given this injection 100, 52, 26 and 17 hours before all the animals were sacrificed. At autopsy, there was gross evidence of leukemia in the 20 leukemic animals. Microscopic examination of small portions of spleen, liver, lymph node, and bone marrow in each animal of this group revealed leukemic infiltration, whereas in no case was there infiltration of skeletal muscle. Certain individual tissues and the remaining carcasses of each group of 5 leukemic and 5 control mice were lumped,³ weighed,

ashed and then assayed for beta ray activity. In this manner, the per cent retention of the dose given, in terms of one gram of whole animal, liver, spleen, lymph node, muscle and bone was determined. The data thus obtained are given in Table II and plotted in Figure 1.

Figure 1 shows that per gram of whole body, leukemic animals take up slightly more phosphorus than normal, but exchange or lose it at about the same rate as normal animals over the period of 100 hours. Skeletal muscle of leukemic animals took up less at first, but later the uptake was approximately normal. It should be noted that there was no leukemic infiltration of the muscle in the leukemic animals. A striking difference, however, is noted in the lymph glands where the uptake by leukemic lymph nodes is approximately three times normal, which is also true of leukemic spleen where the loss of labelled phosphorus is also relatively greater over the period of 100 hours. The curves for bone, which include bone and bone marrow, show that leukemic bone has slightly the greater activity, but the rate of exchange is about the same as normal. The curves for liver are nearly the same for the normal and leukemic animals.

³ Although it is desirable to analyze the tissues of each mouse individually, in these experiments the tissues of 5 animals were lumped since the total weight of the lymph nodes of single normal animals and their uptake of labelled phosphorus are so low that accurate activity analyses are difficult. Three of us (J H L, L W T, and K G S) have confirmed the findings given here in separate experiments not here reported.

TABLE II

Labelled phosphorus in tissues of normal and leukemic mice*
17 HOURS AFTER P³² ADMINISTRATION (NORMAL VS.
LEUKEMIC MICE)

Tissue	Normal or leukemic	Wet weight	Activity in micro- curies per gram	Reten- tion per gram in per cent of dose	Per cent of normal
Lymph node	N	0.126	0.103	2.2	
Lymph node	L	0.327	0.22	4.7	214
Spleen	N	0.640	0.127	2.7	
Spleen	L	3.705	0.385	8.2	302
Liver	N	6.490	0.184	3.84	
Liver	L	9.990	0.189	4.0	104
Muscle	N	4.684	0.085	1.8	
Muscle	L	4.210	0.057	1.22	68
Bone	N	0.682	0.167	3.56	
Bone	L	0.705	0.185	3.96	111
Balance	N	109	0.077	1.64	
Balance	L	104.0	0.081	1.72	105
Total animal	N	121.6	0.084	1.79	
Total animal	L	122.9	0.099	2.1	117

* All activities corrected to January 18 1939

Average retention per normal animal of tagged phosphorus administered 43.5 per cent.

Average retention per leukemic animal of tagged phosphorus administered 52 per cent.

26 HOURS AFTER P³² ADMINISTRATION (NORMAL VS.
LEUKEMIC MICE)

Tissue	Normal or leukemic	Wet weight	Activity in micro- curies per gram wet weight	Reten- tion per gram in per cent of dose	Per cent of normal
Lymph node	N	0.225	0.105	2.23	
Lymph node	L	0.282	0.270	5.77	259
Spleen	N	0.723	0.144	3.06	
Spleen	L	3.550	0.280	6.0	196
Liver	N	6.802	0.178	3.78	
Liver	L	10.860	0.187	4.0	106
Muscle	N	2.745	0.120	2.56	
Muscle	L	3.865	0.076	1.6	63
Bone	N	0.772	0.156	3.3	
Bone	L	0.670	0.199	4.2	127
Balance	N	114	0.070	1.49	
Balance	L	99.5	0.885	1.87	126
Total animal	N	125.2	0.078	1.66	
Total animal	L	118.7	0.95	2.01	121

Average retention per normal animal of tagged phosphorus administered 42 per cent.

Average retention per leukemic animal of tagged phosphorus administered 48 per cent.

TABLE II—Continued

52 HOURS AFTER P³² ADMINISTRATION (NORMAL VS.
LEUKEMIC MICE)

Tissue	Normal or leukemic	Wet weight	Activity in micro- curies per gram wet weight	Reten- tion per gram in per cent of dose	Per cent of normal
Lymph node	N	0.245	0.102	2.18	
Lymph node	L	0.642	0.230	4.88	223
Spleen	N	0.920	0.216	4.6	
Spleen	L	4.600	0.217	4.62	101
Liver	N	5.900	0.159	3.37	
Liver	L	10.358	0.171	3.68	109
Muscle	N	3.730	0.076	1.62	
Muscle	L	2.550	0.086	1.83	113
Bone	N	0.745	0.211	4.5	
Bone	L	0.465	0.232	4.95	110
Balance	N	90.96	0.082	1.74	
Balance	L	78.000	0.089	1.9	109
Total animal	N	104.5	0.087	1.85	
Total animal	L	96.6	0.105	2.24	121

Average retention per normal animal of the tagged phosphorus administered 38.4 per cent.

Average retention per leukemic animal of the tagged phosphorus administered 54.3 per cent.

100 HOURS AFTER P³² ADMINISTRATION (NORMAL VS.
LEUKEMIC MICE)

Tissue	Normal or leukemic	Wet weight	Activity in micro- curies per gram wet weight	Reten- tion per gram in per cent of dose	Per cent of normal
Lymph node	N	0.220	0.109	2.32	
Lymph node	L	0.413	0.200	4.28	185
Spleen	N	0.720	0.110	2.34	
Spleen	L	2.777	0.179	3.82	168
Liver	N	5.042	0.101	2.14	
Liver	L	9.365	0.112	2.39	112
Muscle	N	4.054	0.063	1.34	
Muscle	L	3.642	0.056	1.2	90
Bone	N	0.521	0.206	4.38	
Bone	L	0.486	0.247	5.25	120
Balance	N	101.000	0.053	1.18	
Balance	L	95.92	0.066	1.4	118
Total animal	N	111.500	0.059	1.25	
Total animal	L	112.6	0.073	1.57	125

Average retention per normal animal of tagged phosphorus administered 27.6 per cent.

Average retention per leukemic animal of tagged phosphorus administered 35 per cent.

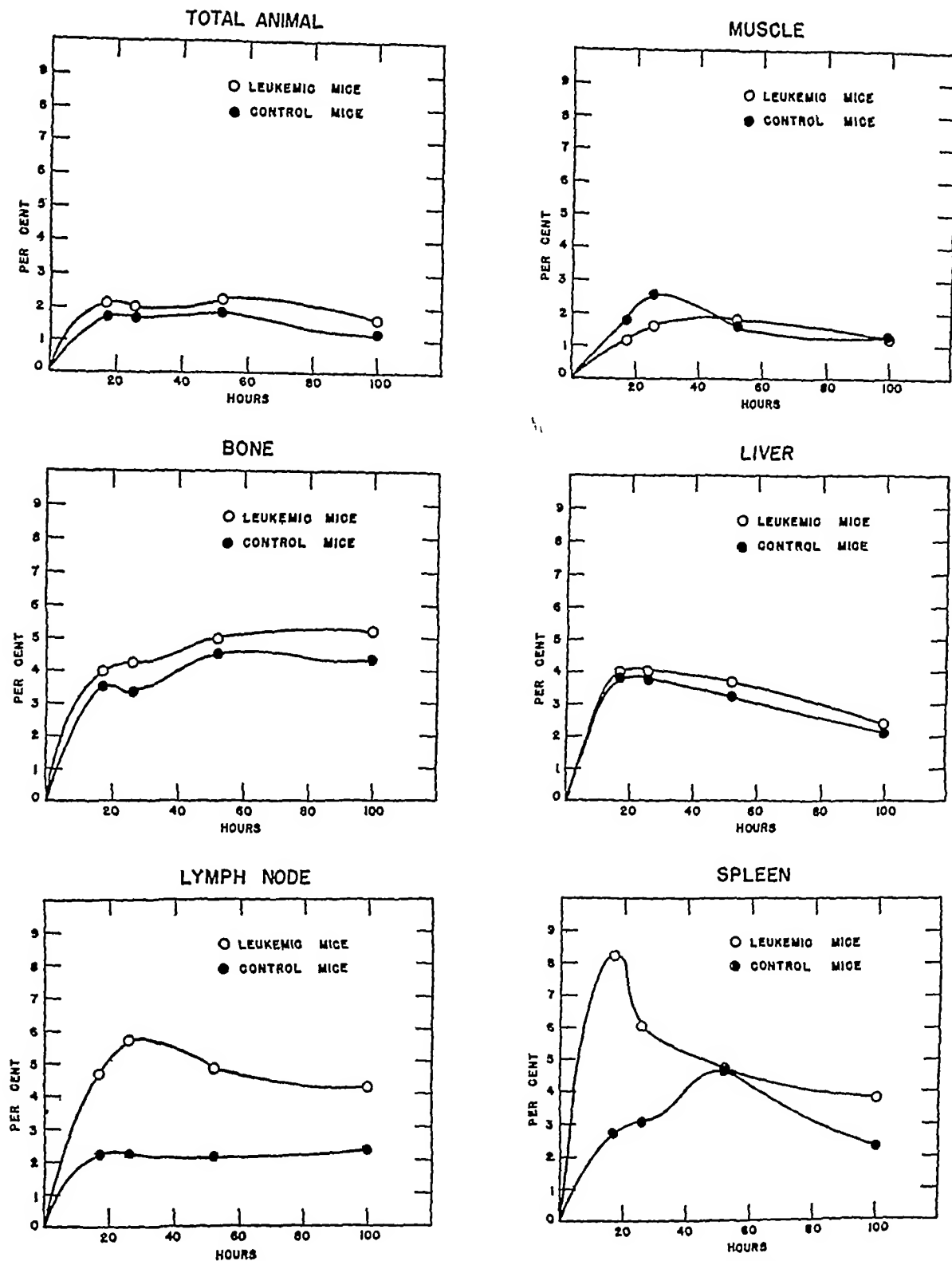
RETENTION OF P^{32} PER GRAM IN VARIOUS TISSUES

FIG 1 RELATIVE EXCHANGE OF RADIOACTIVE PHOSPHORUS IN NORMAL AND LEUKEMIC ANIMALS

Each point represents the tissues from 5 animals. The activities per gram wet weight are plotted against hours after injection of P^{32} in the form of Na_2HPO_4 and they are expressed in per cent of dose per gram of

DISCUSSION AND SUMMARY

The uptake of radio-phosphorus in 20 leukemic and 20 normal animals during the 100 hours before sacrificing has been compared. Gross and microscopic examination of the animals showed that the lymph nodes, the spleens, the livers and the bone marrows of the leukemic animals were infiltrated with leukemic cells, whereas the skeletal muscle showed no infiltration. The average uptake per gram of whole body and bone was only slightly greater in the leukemic animals while the uptake in muscle was slightly less in the leukemic animals. On the other hand, the uptake and exchange in leukemic spleen and lymph gland are strikingly greater than normal. In previous work (1) it was shown that when mice have local lymphomatous tumors the greater uptake and exchange in this tissue occur partially at the expense of whole bone and liver. If the markedly greater uptake of phosphorus in the spleen and lymph nodes of leukemic animals of the present experiment is related to the extensive infiltration of these organs with leukemic cells then one would expect a similar large uptake in other infiltrated tissues such as liver and bone (which includes bone marrow). As noted in the figures the observations in these experiments do not show this. A reasonable explanation would seem to lie in the probability that "stealing" of available phosphorus from bone, liver and muscle has occurred as a result of the large deposition of phosphorus in leukemic tissue generally. Also it is probable that the metabolism of phosphorus in bone marrow is largely masked by the metabolism of phosphorus in cortical and cancellous bone since the latter has such a high content of phosphorus. It seems impossible at the present time to analyze accurately the factors which are concerned in the uptake and exchange of the phosphorus element in these normal and leukemic tissues. We have shown that there is little if any difference in the total phosphorus content of infiltrated or un-infiltrated lymph node, spleen or liver. The uptake or exchange of this element must be associated with the metabolism of the many in-

organic and organic compounds (nucleo-proteins, phospholipids, etc.) and with the building of new tissue, which contains phosphorus as an essential constituent. In the case of leukemic tissue the greater uptake and exchange would seem to be related to first the rapid building of new tissue and second, the greater rate of metabolism of this tissue. It is clear that the uptake of a single dose of phosphorus by any tissue is independent of the total phosphorus content of that tissue. Fractionation of the various organic and inorganic phosphorus compounds in the tissues is being carried out to throw light upon these questions in normal and diseased conditions. From the practical standpoint, it seems important to note that in the leukemic animals the tissues having the highest activities are spleen, bone, lymph node and liver, and these are the tissues predominantly involved in the leukemic process. Unlike x rays which penetrate great distances in tissue, the beta rays from radio-phosphorus penetrate less than a centimeter and thus act locally. Therefore, the relatively high concentration of radio-phosphorus in leukemic tissues gives us a method of somewhat localizing the irradiation to the infiltrated areas, and thus should prove valuable in the therapy of this disease.

WPA assistance (Project Number 10695) is acknowledged, and we also wish to thank the staff of the Radiation Laboratory whose generous cooperation made radio-phosphorus available to us.

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A COMPARISON OF PROCEDURES FOR INCREASING BLOOD FLOW TO LIMBS USING AN IMPROVED OPTICAL PLETHYSMOGRAPH

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Measuring the minute volume of blood flow through an extremity at rest or in response to a physiological stimulus offers a useful means of evaluating the status of its vascular bed. Hewlett and Van Zwaluwenburg (1) used for this purpose a plethysmograph based upon the principle demonstrated by Brodie (2) wherein sudden occlusion of the veins draining the limb produces an increase in limb size, which can be used as a measure of arterial inflow. If a limb is enclosed in a rigid container the increase in volume can be recorded in a variety of ways. Hewlett and Van Zwaluwenburg (3) used air conduction and optical recording, more recently others have used water or water and air conduction with a Brodie bellows, Gad volume recorder or a piston recorder. Experience has shown us that these latter methods have concealed errors which become apparent when replaced by air conduction and optical recording. These are (a) errors in calibration due to a non rigid closure of the open end of the plethysmograph, (b) errors due to involuntary movement of the extremity into and out of the plethysmograph, (c) errors produced by rising intravascular tension secondary to venous obstruction, (d) errors due to the effect of displacement of blood from beneath the collecting cuff. This report concerns itself with a study of these errors, with means of eliminating them and with a comparison of five procedures commonly used to produce dilatation of the vascular bed in the lower extremity.

APPARATUS

A modification of the Hewlett and Van Zwaluwenburg leg plethysmograph (3) was devised which has the following chief advantages (a) greater accuracy in calculation of minute blood flow (b) ease and accuracy of calibration, (c) rigid air tight non-constricting closure of the open end, (d) ease of application.

The plethysmograph (Figure 1) is made of cast

aluminum formed like a boot with a capacity of 6,500 cc.¹ It is bivalved on its long axis and when fitted together about the leg is closed with a rubber gasket. A thin rubber pad (not shown in drawing) is interposed between the sole of the foot and the boot wall upon which it rests. To keep the air surrounding the limb at a constant and at any desired temperature, the plethysmograph is immersed in a water bath. After immersion the boot is anchored firmly by a sliding gate made water tight with a sponge rubber gasket (Figure 1).

The space between the leg and boot wall at its mouth is filled with fast-setting plaster of paris (Figure 1). A cardboard ring supported by a ledge in the boot wall prevents the plaster while in its liquid state from falling into the plethysmograph. Before the plaster becomes firm, the leg is moved from side to side to create a 2 mm space between the skin and adjacent plaster. When the plaster is firmly set the leg is withdrawn 2 to 3 cm and melted Unna paste is applied in a thick layer to the skin surface which is to be in contact with the plaster. The leg is quickly returned to its natural position in the boot the Unna paste (Figure 1) completely filling the crevice between the skin and plaster. A generous application of melted Unna paste applied over the outer surface of the plaster and for a distance of 3 cm upon the skin of the leg completes an air tight rigid closure of the plethysmograph without producing any venous obstruction. The elasticity of the Unna paste permits slight voluntary controlled movement of the limb which relieves the cramplike sensations accompanying prolonged experiments.

Volume changes of the limb within the plethysmograph are recorded optically with a Frank segment capsule joined to the plethysmograph by

¹ The plethysmograph for the foot alone has a capacity of 3,200 cc.

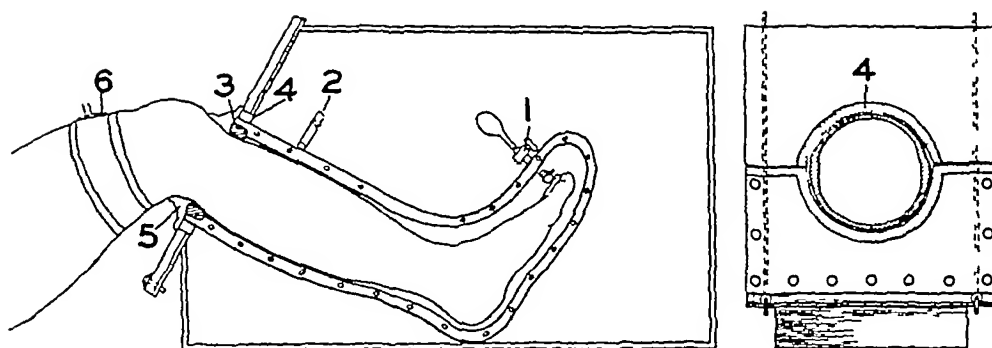


FIG 1 DIAGRAM OF BOOT PLETHYSMOGRAPH

(1) Calibrating stopcock, (2) rubber tube connecting plethysmograph with Frank segment capsule, (3) plaster of paris seal, (4) sponge rubber gasket, (5) Unna paste seal, (6) collecting cuff

a piece of stiff rubber tubing 15 cm long with a bore of 1.2 cm

Sudden obstruction to venous drainage from the limb is accomplished in the conventional manner by a blood pressure cuff (hereafter referred to as the "collecting cuff") 6 cm in diameter (Figure 1) connected to a reservoir containing air at the desired pressure. The moment at which the collecting cuff is inflated is recorded by a Frank segment capsule (Figure 2)

It is necessary (for reasons to be discussed later in the paper) to have the plethysmograph open to the atmosphere when the collecting cuff is inflated and for a period of one second thereafter. This is accomplished as follows. A stopcock is introduced in the tube connecting the pressure reservoir with the collecting cuff and another in a side arm from the tube connecting the plethysmograph with the Frank segment capsule. A solenoid actuated by a time switch op-

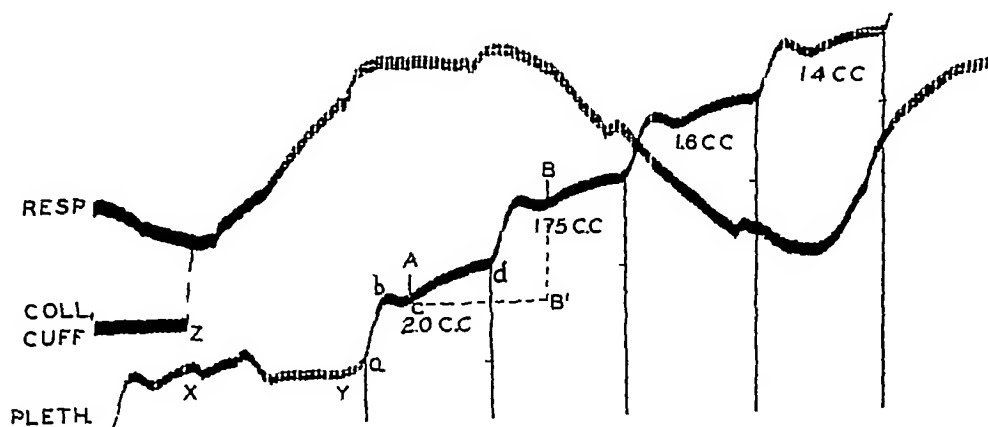


FIG 2 OPTICAL RECORD OF PHASIC BLOOD FLOW IN LEG

Resp Movements of chest wall with down stroke denoting inspiration.
Coll Cuff Pressure changes in the collecting cuff
Pleth Limb volume changes as recorded by the plethysmograph.
Z Denotes moment of collecting cuff inflation.
X-Y Interval during which plethysmograph is open.
a-b Rapid arterial inflow phase.
b-c Arterial outflow phase.
c-d Slow arterial inflow phase.
A-B Cycle from which blood flow calculation is made.
B-B' Ordinate representing net arterial inflow for cycle A-B
 Numbers represent value in cc. of the net arterial inflow for each successive cycle.

erates both of these stopcocks simultaneously. When the timing switch is closed the solenoid opens both stopcocks. Thus the plethysmograph is open (Figure 2X) to the atmosphere at the time the collecting cuff is inflated. When the timing switch opens one second later the solenoid is released and a spring mechanism closes the plethysmograph stopcock, thus allowing the volume changes of the limb to be recorded (Figure 2Y).

Calibration of the plethysmograph is accomplished by injecting or withdrawing a measured volume of fluid through a stopcock (Figure 1) into a small rubber bag within the plethysmograph. This must be done with the extremity *in situ* and with the arterial inflow completely obstructed by pressure in the collecting cuff.

The natural frequency of the apparatus in situ ranges from 50 to 100 per second over such a range of sensitivity that a 1 cc. increase in the fluid content of the plethysmograph produces a light beam excursion of 2 to 10 cm. at a projection distance of 1.5 meters.

Immobility of the limb inside of the plethysmograph is checked at the beginning of and during each experiment by completely occluding the artery and observing whether or not any variation in the plethysmograph volume occurs synchronously with breathing. When involuntary movement of the limb into and out of the plethysmograph is noted it is easily abolished by placing a wide gauze bandage across the superior margin of the patella and tying each end to the water bath thus anchoring the limb without producing any venous obstruction.

Respiratory excursions of the chest wall are recorded by a Frank segment capsule connected with a pneumograph. The volume of the limb contained in the plethysmograph is determined by displacement. Brachial arterial blood pressure determinations are made by a mercury manometer and auscultation immediately after each measurement of blood flow.

EXPERIMENTAL PROCEDURE

For these studies the subject lies upon his back in a Gatch bed with the hip and knee flexed about 35° and the head and shoulders slightly elevated, thus bringing the heart and the extremity being measured approximately to the same level. This position is chosen because it pre-

vents the development of high venous pressure in the limb and is known to be the position best tolerated with comfort over a period of several hours. To obtain standard experimental conditions the following requirements are observed: (a) severe exercise is prohibited for 24 hours prior to a study; (b) smoking is not allowed within the hour preceding and during a study; (c) unnecessary talking and other disturbing influences are avoided during the observation; (d) studies are begun 3 to 4 hours after the last meal; (e) the subject, clad in trunks, reclines quietly for 45 minutes in a room kept at a constant temperature between 28 and 30° before being studied; (f) a lapse of 30 minutes is allowed after completing the application of the boot plethysmograph before records are taken. This allows the flow of blood to assume a constant level and the heat in the setting plaster and melted Unna paste to dissipate.

METHOD OF CALCULATING BLOOD FLOW

A typical record is shown in Figure 2. The upper curve denotes respiratory excursions of the chest wall, inspiration being represented as a down stroke. The moment of inflation of the collecting cuff is signalled by the sharp rise (Z) of the second curve. The lower curve (time marker equals 0.4 seconds) is a record of the variations in volume of the limb contained in the plethysmograph. It is noted that with each pulse there is a rapid increase in limb volume (a-b) followed by a small decrease (b-c) after which there is a gradual further increase (c-d). In terms of blood flow each pulse shows an initial large inflow followed by a small outflow which in turn is followed by a gradual inflow of blood. Actually each pulse (Figure 2, a-d) represents a net inflow of arterial blood. That portion of the cycle (b-c) representing outflow merits more detailed discussion later.

It is apparent from these variations during a single pulse that the net inflow (a single complete cycle) is the smallest unit that can be used for measuring blood flow. Methods using the gradient of any portion of a cycle for calculating blood flow are distinctly in error. As the curve rises, each successive beat forces a smaller net volume of blood into the leg because of rising venous resistance. In this curve (Figure 2) the net flow per successive beat is 2.0, 1.75, 1.6, 1.4 cc. This fact introduces an error in all procedures using the volume of several successive beats to calculate minute blood flow. To avoid the error introduced by a rising venous resistance, the first complete cycle following inflation of the collecting cuff should be used as the unit most nearly representing true arterial inflow. Use of this cycle, however, is complicated by the fact that the collecting cuff as it is inflated squeezes an appreciable volume of blood from the vessels underlying it into the tissues contained in the plethysmograph. The effect of the displaced blood on the limb volume cannot be differentiated by the plethysmograph from the effect of normal arterial inflow. Optical recording methods also show that this effect cannot be avoided by varying the size or distances of the collecting cuff from the plethysmograph as has been supposed by

investigators using less sensitive, low frequency apparatus. Since this action of the collecting cuff cannot be prevented, the first cycle following the termination of its effect becomes the earliest available cycle for measuring blood flow. The time required to complete the displacement of blood from beneath the collecting cuff is determined by completely occluding the arterial inflow to the leg with an inflated cuff on the mid-thigh and then inflating the collecting cuff in its usual position with a pressure of 80 mm. of mercury. The increase in limb volume within the plethysmograph (Figure 3, A-B) re-

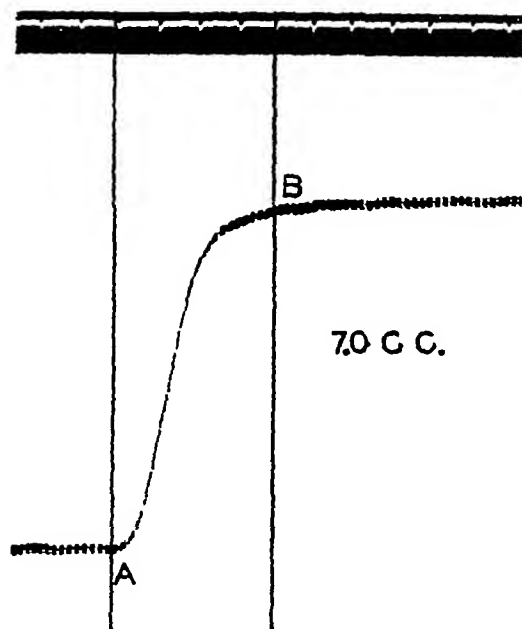


FIG 3 DISPLACEMENT OF FLUID FROM BENEATH COLLECTING CUFF

A-B Magnitude of and time required for displacement of fluid from beneath collecting cuff

sulting from displacement of fluid by the collecting cuff, even when of large magnitude, never requires more than 1.2 seconds. Consequently, the undesirable effect of the collecting cuff can be circumvented by measuring the increase in limb volume for one pulse cycle beginning at a point (Figure 2-A) 16 seconds after inflating the collecting cuff, regardless of the place in the cycle at which this point falls. In order to nullify the error that might be introduced by a sinus arrhythmia, the duration of this cycle (Figure 2, A-B) must be equal to the average of several cycles recorded during the flow measurement. The ordinate value B-B¹ representing the net arterial inflow for one cycle is converted to cubic cm. and the net arterial blood flow is expressed in the conventional manner of cc. per 100 cc. limb substance per minute.

The sudden increase in limb volume produced by the massaging action of the collecting cuff is not recorded (Figure 2, X-Y) by the simple expedient of having the plethysmograph open to the atmosphere during the time

this event occurs. This is accomplished by the apparatus described earlier.

In addition to errors resulting from inadequate apparatus, imperfections in technique may be introduced by variations in the individual being studied. Observations show that records taken more frequently than every 2 minutes result in progressively smaller blood flows. Apparently sufficient time (2 minutes) must be allowed after each test for the accumulated blood to leave the tissues in order to restore normal dynamic conditions.

TABLE I

Constancy of leg-foot blood flows taken at 2-minute intervals

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
9.36	8.0	104/66	71	28	45	43	37	80
9.38	7.4	104/66	65	28	45	43	37	80
9.40	7.6	104/66	73	28	45	43	37	80
9.42	7.4	104/66	71	28	45	43	37	80
9.44	7.9	104/66	71	28	45	43	37	80

Blood flow is expressed in cc per minute per 100 cc leg tissue

Blood pressure and cuff pressure are expressed in mm of mercury

All temperatures are expressed in degrees centigrade

TABLE II

Effect of blood pressure upon blood flow

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
11.00	6.7	116/70	69	28	28	28	37	75
11.03	6.6	116/70	57	28	28	28	37	75
11.12	6.8	116/70	58	28	28	28	37	75

HANDS IMMERSSED IN WATER AT 11 DEGREES CENTIGRADE FOR 5 MINUTES

11.20	8.4	130/82	68	28	28	28	37	75
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It scarcely seems necessary to point out the absolute necessity of blood pressure observations for interpreting variations in blood flow. Nevertheless there is a conspicuous absence of such data in published reports concerning this subject. Table II shows the influence of blood pressure change upon the blood flow in the foot. It is apparent also from the contour of the phasic flow that if the heart rate is increased, even though there be no change in systolic or diastolic pressure, there may be an increase in the peripheral blood flow since it is the latter part of the cycle that is shortened.

Table III shows that the pressure in the collecting cuff can vary over a wide range with but little variation in the recorded blood flow.

TABLE III

Effect of lowering the collecting cuff pressure upon the recorded blood flow

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Foot temperature	Oral temperature	Cuff pressure
8:00	3.7	120/72	68	28	28	28.5	37	80
8:04	3.9	120/72	69	28	28	28.5	37	60
8:07	3.8	120/72	71	28	28	28.5	37	42
8:09	3.4	120/72	68	28	28	28.5	37	35
8:12	3.3	120/72	70	28	28	28.5	37	25
8:15	2.8	120/72	70	28	28	28.5	37	15

The arterial backflow

The record shown in Figure 2 reveals a brief reduction in limb size, *b-c* which is due to an outflow of blood occurring in the presence of a pressure of 80 mm. of mercury in the collecting cuff. The only channels by which blood can leave are the superficial and deep veins of the leg, the bone canals and the arteries. Escape via the veins under the inflated collecting cuff is excluded by *a priori* considerations and also by the fact that it occurs during the first beat after inflating the cuff *i.e.* at a time when the pressure in the veins cannot conceivably overcome that produced by the collecting cuff. Furthermore, it varies greatly in magnitude in different individuals and in the same individual under varying conditions, in fact, it may be entirely absent. Outflow of blood via vessels of the bones in the foot and leg is not plausible since these vessels join more superficial veins before they reach the collecting cuff. We agree with Hewlett and Van Zwaluwenburg (3) that by exclusion, the only conclusion tenable is that the reduction in limb size (Figure 2, *b-c*) represents arterial backflow. We believe it to be a phenomenon normally present in unimpeded blood flow in the extremities.

The forces responsible for arterial backflow arise in the following manner. The discharge of blood from the left ventricle into the aorta initiates a pressure wave which is rapidly propagated along the aorta and into its branches. This pressure wave accelerates the flow of blood in the regions through which it travels, producing a wave of accelerated blood flow. Upon reaching the terminal portions of the arterial tree, the momentum of this accelerated column of blood for a brief period forces more blood into the small

arteries and arterioles than can flow from them into the capillaries. Consequently, for a moment blood accumulates in the distal end of the arterial tree. As the blood accumulates, it also decelerates and a portion of its energy of flow is converted into energy of lateral pressure. As a result, the pressure of the accumulated blood in the small arteries for a brief moment exceeds that of the blood in the larger arteries, and arterial backflow occurs.

The pressure relationships necessary to produce this arterial backflow have been found to exist in the limb of the dog by Hamilton and Dow (4) and in the lower extremity of the human by Hamilton, Woodbury and Harper (5). The demonstration of backflow in arteries as far distal as those of the foot requires that these vessels must be included in the oscillating arterial system the reflected waves (not only pressure but also actual fluid displacement) from which influence the contour of the arterial pulse in all segments proximally located. This view has previously been suggested by Hamilton (4) on the basis of arterial pressure measurements.

Figure 4 shows the phasic flow of blood at rest (*A*) and after the application of local heat (*K*). The backflow (*B-C*) present normally is abolished (*D-E*), presumably due to dilatation of the arterioles and a consequent lessening of blood accumulation in the small arteries with each heart beat.

Figure 5 shows the phasic and net arterial blood flow of the foot before (*H*) and after (*I*) inhalation of amyl nitrite and before (*J*) and after (*K*) sacral diathermy. Each of these procedures results in an increase of the initial inflow phase (*A-B*), and an increase in the arterial backflow phase (*B-C*) with a reduction in the net arterial inflow (*D-E*). In spite of an increase in heart rate, the minute flow is reduced in each instance. Because of the increase in the initial arterial inflow phase (*A-B*) an oscillogram record of these two observations would be erroneously interpreted as demonstrating an increase in net arterial blood flow. Hewlett and Van Zwaluwenburg (3) called attention to this same error in volume pulse recorders (oscillometers) in 1913. We raise the point again to emphasize an important observation which has been overlooked or forgotten by numerous investigators.

Resting blood flow studies

When the foot and leg are exposed to a temperature of 45° C for 30 minutes extraneous influences on the minute volume of blood flow are minimized. Table I shows that under these conditions the minute volume of blood flow does not deviate more than 6 per cent from the mean. A glance at the resting values for blood flows in Tables III, IV, V, VI, VII and VIII reveals only

slightly larger variations in the unheated leg, suggesting that the foot and leg (more so than the foot alone) represent a more stable vascular bed than heretofore believed.

The same tables also show the well-known large variations in values for resting blood flows obtained in different individuals, even when determined under quite similar conditions. Resting blood flow determinations in the same individual

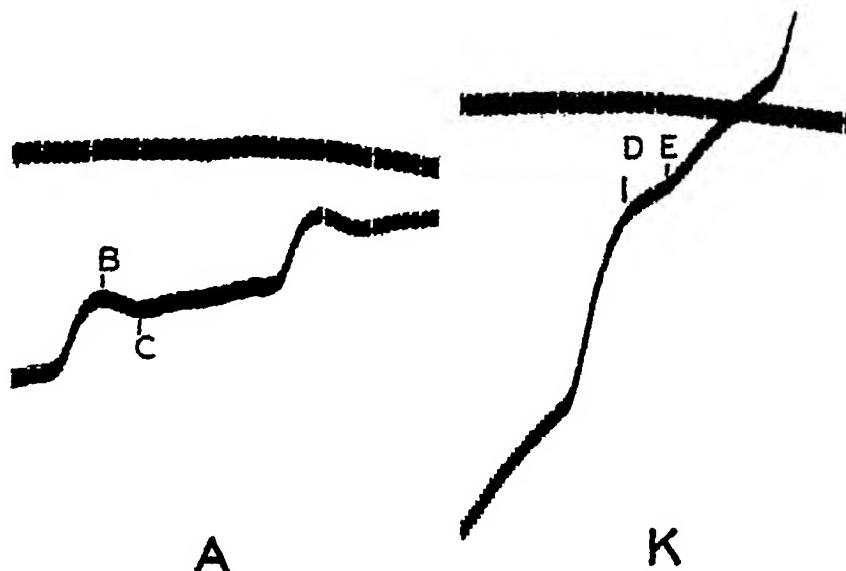


FIG 4 THE PHASIC BLOOD FLOW *A* BEFORE AND *K* AFTER LOCAL HEAT IN THE SAME INDIVIDUAL

B-C Phase of arterial backflow
D-E Absence of arterial backflow

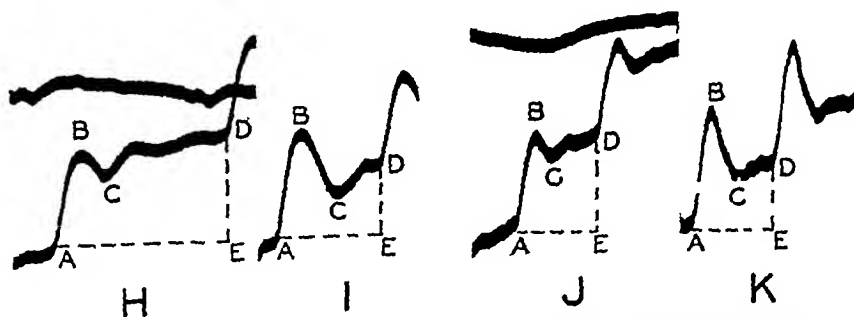


FIG 5 THE EFFECT OF AMYL NITRITE AND SACRAL DIATHERMY UPON PHASIC BLOOD FLOW

H Normal resting flow
I Same individual after inhaling amyl nitrite.
J Normal resting flow
K Same individual after sacral diathermy
A-B Rapid arterial inflow phase.
B-C Arterial backflow phase
C-D Slow arterial inflow phase
D-E Net arterial inflow

TABLE IV

Effect of the heat reflex on leg-foot blood flow

RESTING BLOOD FLOW

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
3.31	1.8	106/70	82	29.8	30	30	37.3	70
3.38	2.0	106/70	82	29.8	30	30	37.3	70

3.45—UPPER EXTREMITIES IMMERSSED IN WATER AT 45 DEGREES CENTIGRADE

4.22	3.8	108/72	89	29.8	30	30.5	37.3	70
4.26	3.9	108/72	92	29.8	30	30.5	37.3	70
4.36	4.2	108/72	89	29.8	30	30.5	37.3	70

4.45—WATER BATH OF PLETHYSMOGRAPH CHANGED TO 45 DEGREES CENTIGRADE

5.17	6.9	102/68	92	30	45	44	37.3	70
5.19	6.9	102/68	89	30	45	44	37.3	70
5.23	6.8	104/72	89	30	45	44	37.3	70

TABLE V

Effect of static nerve block on leg-foot blood flow

RESTING BLOOD FLOW

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
2.40	3.0	108/72	80	27	27	28	37	75
2.45	2.9	112/70	80	27	27	28	37	75
2.50	3.2	112/70	80	27	27	28	37	75

SCIATIC NERVE BLOCKED WITH 10 PER CENT PROCAINE (COMPLETE MOTOR AND SENSORY)

3.45	6.0	112/70	71	27	27	30	37	75
3.57	5.5	108/70	67	27	27	30	37	75
4.00	6.2	108/70	71	27	27	30	37	75
4.10	6.2	108/70	74	27	27	30	37	75

4.30—WATER BATH OF PLETHYSMOGRAPH CHANGED TO 45 DEGREES CENTIGRADE

5.05	6.3	108/66	77	27	45	44	37	70
5.15	6.3	108/66	76	27	45	44	37	70

on different days under non basal conditions (Table VIII) show no constancy whatever but under strictly basal conditions (Table VIII) the same individual shows a remarkably constant resting blood flow. This emphasizes the importance of observing such basal conditions when making blood flow determinations on the same individual for comparative purposes.

Minute blood flow in response to vasodilating procedures

The response of the peripheral vascular bed to vasodilating procedures frequently furnishes valuable clinical information regarding their capacity for further dilatation. We have compared the effect of five such procedures in normal healthy young men and have studied the constancy of the reaction of the one producing the maximum vasodilatation. The methods studied are (a) heat reflex method of Gibbon and Landis (6), (b) sciatic nerve block (7), (c) spinal anesthesia (8)

TABLE VI

Effect of spinal anesthesia on leg-foot blood flow

RESTING BLOOD FLOW

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
3.07	2.1	112/72	67	28	28	28.5	37.2	70
3.09	2.3	112/72	67	28	28	28.5	37.2	70

120 MG. OF NOVOCAIN CRYSTALS INJECTED INTRATECALLY AT 2ND LUMBAR WITH COMPLETE MOTOR AND SENSORY PARALYSIS TO AND INCLUDING D12

4.04	2.3	102/72	57	28	28.5	29.5	37	70
4.06	2.5	102/72	61	28	28.5	29.5	37	70

4.10—UPPER EXTREMITIES IMMERSSED IN WATER AT 45 DEGREES CENTIGRADE

4.44	4.0	108/72	66	28	29	29	37.2	70
4.49	4.2	108/72	63	28	29	29	37.2	70

TABLE VII

Effect of sacral diathermy on foot blood flow

RESTING BLOOD FLOW

Time	Blood flow	Blood pressure	Heart rate	Room temperature	Bath temperature	Boot temperature	Oral temperature	Cuff pressure
2.32	4.0	104/60	64	28	28	28	37.2	65
2.34	4.2	110/60	68	28	28	28	37.2	65

DIATHERMY 1500 MILLIAMPERES APPLIED OVER SACRUM FOR 30 MINUTES

3.30	2.7	106/54	67	28	28	29	37.8	65
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3.35—WATER BATH OF PLETHYSMOGRAPH CHANGED TO 45 DEGREES CENTIGRADE

4.12	9.7	106/60	67	28	45	44	37.6	65
4.26	11.7	110/64	64	28	45	44	37.6	65

TABLE VIII
Constancy of effect of local heat under basal and non basal conditions
 NON-BASAL BLOOD FLOW

Subject	Resting blood flow					Blood flow after local heat			
	Date	Time	Blood flow	Blood pressure	Heart rate	Time	Blood flow	Blood pressure	Heart rate
W Z	January 10, 1939	8 12 P M	5 6	112/72	72	8.56 P M	6 3	112/72	60
		8 15	5 2	112/72	70	8.58	6 2	112/72	68
						9.05	7 2	112/72	70
W Z	January 11, 1939	8 28 P M	7 2	116/76	71	9.33 P M	8 0	108/66	71
		8.32	6 7	116/76	68	9.36	7 4	108/66	71
						9 40	7 6	108/66	7 3

BASAL BLOOD FLOW

Subject	Date	Time	Blood flow	Blood pressure	Heart rate	Time	Blood flow	Blood pressure	Heart rate
W Z	January 13, 1939	9.30 A M	2 1	98/66	41	10.23 A M	4 9	98/64	60
		9.32	2 1	94/64	50	10.25	5 0	98/64	53
		9 41	2 8	94/64	58	10.35	6 4	102/68	63
						10.37	5 6	102/68	60
W Z	January 14, 1939	9 11 A M	2 2	110/70	57	9 48 A M	4 0	108/64	62
		9 13	2 1	110/70	55	10.07	5 4	110/60	62
		9 16	2 1	110/70	55	10 15	5 2	110/60	64

AFTER BREAKFAST BUT NO MUSCULAR ACTIVITY

W Z	January 15, 1939	10 40 A M	3 6	110/68	60	11 24 A M	5 0	108/70	60
		10 45	3 7	110/68	62	11 40	5 6	108/70	67
						11 42	5 1	104/76	61

(d) sacral diathermy (10), (e) local heat applied for 30 minutes at 45° C

Reflex heat Table IV shows the typical reaction obtained in 1 of 5 subjects resulting from immersion of the hand and forearm of both upper extremities in water at 45° C for 30 to 45 minutes. In each individual this procedure produced an unquestionable increase in blood flow above the resting level. When this was immediately followed by local heat applied to the leg in the plethysmograph, a further marked increase was obtained in each instance.

Sciatic nerve block Blocking the vasomotor fibers in the sciatic nerve (Table V) produced in each of 5 subjects a marked increase in blood flow as compared to the resting minute volume. In no case did reflex heat or local heat in the presence of sciatic nerve block produce a further increase in blood flow.

Sacral diathermy Of 3 subjects studied by applying diathermy (1500 M A) to the sacral area, 2 showed an actual decrease and 1 a very

slight increase in blood flow (Table VII). All 3 responded with an increase in blood flow upon the application of reflex heat immediately after the diathermy.

Spinal lumbar anesthesia Table VI shows the effects upon blood flow in the foot and leg produced by spinal anesthesia from T-12 down. Complete motor and sensory paralysis was present in all 3 of the subjects so studied, 1 other having the same level of anesthesia and the third with anesthesia extending up to T-10. In no case did we find any evidence of an increase in blood flow as compared to the resting level. In each case heating the upper extremities produced an increase in blood flow in the presence of the motor and sensory paralysis due to the spinal anesthesia.

Local heat It is apparent from the foregoing tables that the maximum dilatation occurs in response to local heat and to sciatic nerve block.²

² We realize that other procedures such as muscular exercise and reactive hyperemia might cause a much greater vascular dilatation but, for obvious reasons, they

In the 5 subjects studied our observations show that local heat and sciatic nerve block, when applied separately, yield the same blood flow response per individual within narrow limits, providing the conditions under which the measurements are done are comparable.

If local heat is to be used as the stimulating agent, the constancy of its response must be known. A series of studies on the same individual (Table VIII) shows that under non basal conditions after a day of ordinary activity the response to local heating is fairly constant. This equality of response, however, is conditioned by one very important observation, as shown in Table VIII. The same subject was studied on 2 days under strictly basal conditions and on the third day under basal conditions so far as activity was concerned but following the ingestion of a light meal. On all 3 days the response to local heat was in very close agreement but of significantly less magnitude than after a day of muscular activity. Apparently muscular activity conditions the response of the peripheral vascular bed to the stimulus of local heat. Studies made upon the same individual on different days for comparative purposes must take this into account. It is preferable to do all measurements under strictly basal conditions.

DISCUSSION OF RESULTS OF DILATING PROCEDURES

We conclude that local heat for 30 to 45 minutes at 45° C can be substituted to advantage for the somewhat more formidable procedure of sciatic nerve block to produce maximal peripheral vascular dilatation at least in normal individuals. The application of this conclusion to diseased vascular beds remains to be tested. It is conceivable that vessels which are damaged or under the control of an abnormal autonomic nervous system may not have the same response to local heat as those in normal individuals. We also noted that the blood pressure and heart rate fluctuate more in response to local heat than to sciatic nerve block.

Our results with spinal anesthesia appear to be at variance with those of Morton and Scott

would not be suitable agents for studying diseased vascular beds. Moreover the blood pressure and heart rate changes accompanying exercise and the difficulty of controlling the amount and rate of work done would seriously complicate the interpretation of the results obtained.

(8) and of Brill and Lawrence (9) who report an increase in blood flow in the foot under similar conditions of spinal anesthesia. An elevation of foot skin temperature was used by them as a measure of increase in blood flow. A possible explanation of our differences might be found in the fact that their subjects were studied in a room with a temperature much lower than ours so that the blood flow of their subjects would be expected to be much less than that of our subjects at the beginning of the experiment. In fact the skin temperature of our subjects was 31 to 32° C on the dorsum of the foot at the beginning of our experiments; a skin temperature virtually equal to that obtained in the subjects of Morton and Scott (8) after spinal anesthesia. For this reason it appears possible that our subjects at rest before spinal anesthesia had blood flows essentially as great as the subjects of Morton and Scott after spinal anesthesia. Such evidence implies that spinal anesthesia does not give complete vasomotor nerve block. This implication finds support in the fact that reflex heat produces an increase in blood flow in the presence of complete skeletal motor and sensory anesthesia. Since spinal anesthesia does not produce an increase in blood flow equal to that resulting from sciatic nerve block, and since reflex heat is effective in the presence of spinal anesthesia it is reasonable to assume that at least a part of the fibers mediating vasomotor impulses to the lower extremity is intact in its presence. Of the various explanations that might be offered for this situation none have sufficient experimental evidence supporting them to warrant their exposition.

In regard to the use of skin temperatures as a means of studying variations in blood flow, two observations merit attention. We have measured the blood flow in many subjects with skin temperatures of 32 to 33° C, finding values of only 2 cc. per 100 cc. per minute. In other words a relatively small blood flow will sometimes maintain a normal skin temperature. Also 1 subject having a foot skin temperature of 35° C with a blood flow of 3 cc. per 100 cc. per minute showed a further rise of only 1° C (to 36° C) when the blood flow increased to 100 cc. per 100 cc. per minute following sciatic nerve block. These observations suggest that skin temperature deter-

minations mirror the changes in blood flow up to a point beyond which the blood flow may increase greatly with but little further rise in skin temperature

Our results with sacral diathermy do not support those reported by de Takats (10) who based his evidence for an increase in blood flow through the foot upon skin temperature determinations. Skin temperature studies are in this particular instance very untrustworthy since the body temperature is elevated (1°C or more) and also since the blood flowing to the skin of the leg may be warmed as it flows through the pelvis via the iliac arteries. The actual decrease obtained in 2 subjects may be due to dilatation of visceral vascular beds in the lower abdomen with a consequent shunting of blood into these areas.

SUMMARY AND CONCLUSIONS

1 Despite many advantages of the Hewlett-Van Zwaluwenburg plethysmographic method for determining blood flow in the limbs, this procedure—and even more its modification by subsequent workers—has a number of hidden faults which lead to inaccuracies in the estimation of flow under different conditions. Among these are the yielding diaphragm surrounding the limb and closing the plethysmograph, displacement of fluid into the plethysmograph by inflation of the collecting cuff, and lack of sensitivity of the entire apparatus.

2 A boot plethysmograph is described which is easy to apply and calibrate, the open end of which is sealed around the leg without constriction by the use of plaster of paris and Unna paste, and which is comfortable for prolonged periods of study. The apparatus is connected to a Frank segment capsule giving the entire system frequencies up to 100 per second.

3 To obviate the artefacts incurred through displacement of fluid into the plethysmograph by the inflated cuff, an arrangement of stopcocks is provided by which the collecting cuff is inflated while the plethysmograph and recording capsule remain open to the atmosphere, but exactly one second later it is automatically closed and the volume changes are recorded. The rise of a full pulse beat starting exactly 1.6 seconds after inflation is used to measure the net flow per cycle. From this the volume flow per minute per 100 cc

of leg substance is calculated in the conventional manner.

4 The phasic arterial blood flow of the leg recorded in this way for normal subjects at rest in a warm room (28° to 30°C), shows three distinct variations during each cycle: (1) a rapid systolic forward flow, (2) a slower smaller and variable systolic backflow, (3) a slow forward flow during diastole. The net arterial inflow depends not only on 1 plus 3 but also upon the amount subtracted by 2. All of these vary under different circumstances. Since only the amplitude (phase 1 above) of the pulse volume is recorded by plethysmographs or oscillograms, these methods give no quantitative estimate of changes in volume flow, and under some conditions they do not even show the correct directional changes.

5 The resting flow determined by our procedure remains constant within approximately 6 per cent of the mean during repeated determinations within an hour. It varies greatly in different individuals or in the same individual from day to day unless strictly basal conditions are observed.

6 A study of various procedures suggested for promoting a maximal blood flow in the leg shows that direct application of heat and sciatic nerve block are the most efficacious. Application of heat to both upper extremities (reflex heat) produces an effect only about one-half as great. We were unable to detect any increase in blood flow following effective spinal anesthesia or sacral diathermy.

7 Other procedures which affect heart rate or blood pressure significantly influence blood flow in a variable manner. Amyl nitrite, for example, increases the amplitude of oscillations but often causes an actual decrease in volume flow.

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THE DETERMINATION OF THE GLOMERULAR FILTRATION BY THE ENDOGENOUS CREATININE CLEARANCE¹

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Since Smith (34) and his collaborator (31) and Miller and Winkler (20) have shown that exogenous creatinine is partially excreted by the renal tubules in man, Rehberg's method (28) for the determination of the glomerular filtration has to be questioned. The interest should have been directed towards the determination of the endogenous creatinine but the discussion as to whether creatinine as such is normally present in plasma, and the absence of an adequate method for the determination of creatinine, delayed investigations upon this subject.

The doubts as to whether plasma creatinine exists or not were finally refuted by the work of Miller and Dubos (19) who developed a specific enzymatic method for the determination of creatinine. They found that in normal individuals creatinine constitutes 80 to 100 per cent of the chromogenic material in tungstic acid filtrates of plasma. In tungstic acid filtrates of plasma from uremic patients there may be large amounts of non-creatinine chromogenic material. This method is very important for scientific research but is unfortunately not applicable in clinical practice.

From another point of view Popper, Mandel and Meyer (26) developed a method for the determination of creatinine based on Jaffe's principle which is said to be perfectly specific and very sensitive. The normal values obtained in plasma by this method are between 0.5 and 1.0 mgm. per cent, while those found by Folin's method (10) are 1 to 2 mgm. per cent. There is reason to believe that this difference is caused by the fact that the picric acid Popper uses for the precipitation of the proteins precipitates the other chromogenic substances as well. Ferro-Luzzi (8) found that the values of plasma creatinine obtained by Somogyi's (37) zinc precipitation of the proteins were below those obtained by Folin and Wu's (11) tungstic acid precipita-

tion. Another reason for the difference between the results is that Folin used the colorimeter which shows these slight differences of color only imperfectly while Popper found his values by the photometer.

Popper and Mandel (25) were the first to apply this method for the determination of glomerular filtration extensively. Only comparatively few previous publications have appeared on the determination of the endogenous creatinine clearance (9, 12, 13, 18). As Popper and Mandel's values on the average, are below those of the authors who employed creatinine administration they conclude that this method shows the real filtration. They made some experiments with phlorizin and with xylose to prove that no secretion of creatinine takes place in the tubules. These few experiments, however, do not prove much as the doses of phlorizin were far too small to inhibit an eventual secretion of creatinine. On the other hand the experiments with xylose are very doubtful since, according to Shannon and Smith (33) the xylose clearance lies 25 per cent below the glomerular filtration, so that a comparison of the excretion of xylose and creatinine can only be made if this fact is taken into consideration.

A method which employs endogenous creatinine has the following advantages. Without the application of the expensive creatinine the determination can be performed more easily and at considerably lower costs. During the time of the test the level of the plasma creatinine remains constant and only one blood sample has to be drawn. The level of creatinine in plasma is very constant and varies only within narrow limits. Therefore it is very important for clinical purposes to know definitely whether Popper's simple and easily applicable method allows an exact determination of the glomerular filtration. The scope of our observations has been to throw light on this question.

¹A previous report of this work was given in the Bulletin de la Faculté de Médecine d'Istanbul (40).

THE DETERMINATION OF THE ENDOGENOUS CREATININE CLEARANCE BY POPPER AND MANDEL'S METHOD (25)

1 Preliminary remarks on the method

The determination of the plasma creatinine is performed by a macromethod for which 4 cc oxalated plasma are used. The protein is precipitated directly by picric acid such as that employed for Jaffe's reaction, so that the dilution of the blood is kept within rather narrow limits. The comparison of color after the alkalization is made in a photometer at as great a depth of layer as possible. With this method it is possible to get exact double determinations even at very low concentrations of plasma creatinine. In normal persons the values are between 0.5 and 1.0 mgm per cent. Values between 1.0 and 1.15 mgm per cent are dubious, those above 1.15 mgm per cent are certainly pathological.

After preservation for from 1 to 2 days in the refrigerator the result does not change, whereas if kept in storage for a longer time, the content of creatinine decreases by about 10 to 40 per cent. The addition of as much as 400 mgm. per cent glucose has no influence on the level of creatinine, higher concentrations of glucose were not added. Acetone and acetoacetic acid, however, increase the depth of color considerably and interfere with the determination of creatinine. β -hydroxybutyric acid was not examined. For this reason patients with acetonuria were excluded from the tests. Some drugs seem also to produce the Jaffe reaction, e.g. salicylic acid given in great quantities. A detailed examination of this point was not made.

In order to avoid such interference and also the probable influence of ingestion of food, all tests are made in the morning on fasting subjects. To get control of regular diuresis it proved practical to collect the urine in two separate hourly specimens in the manner which Möller, McIntosh, and Van Slyke (21) recommended for the urea clearance. If there is a great difference between the quantities of the specimens collected during the two measured periods of about the same length, the test is discontinued. The urines of the two periods, having been measured exactly and mixed, are analyzed and so is the blood sample drawn after the first hour. The analytical methods are briefly described at the end of this article.

2 Comparison between the endogenous creatinine clearance and the inulin clearance

The real answer to the question as to whether a substance fulfills the conditions for the determination of glomerular filtration or not will result from the comparison of the clearance of this substance with the inulin clearance. Inulin alone appears to measure glomerular filtration in man as well as in all the animals examined so far (14, 29, 34). A comparison of the endogenous creatinine clearance with the inulin clearance has only been made in a few cases by Miller and Winkler (20) with the reliable enzymatic method for the determination of creatinine. They found that in most normal persons the clearance of endogenous creatinine equals the inulin clearance. To determine whether the results of Popper's simple method coincided with the inulin clearance we examined 17 cases in forty-two separate periods.

According to Smith, Goldring and Chasis (36) and Miller and Winkler (20) the inulin was introduced by intravenous infusion in order to obtain a constant plasma level. The collection of urine by an indwelling catheter, washing the bladder with sterile saline and limiting the periods, is done as described by Smith, Goldring, and Chasis (36).

For the repeated drawing of the blood an Amiot needle was employed according to Ottenstem (22). After one puncture blood may be drawn as often as necessary during the whole period. The production of pure inulin was very difficult at first. The first samples of a preparation of Merck were very well tolerated, but another preparation of the same origin caused a serious febrile reaction. After repeated recrystallization from hot water and treatment with charcoal we succeeded in getting a preparation which caused a slight febrile reaction only in half of the cases. Since the filtration is always made through a Seitz E. K. filter (35), the inulin is always tolerated without any reaction. The solutions for the infusion are freshly prepared with 0.9 per cent NaCl in double distilled water and are boiled once more for a minute after the filtration through the sterile Seitz filter. With a new colorimetric method for the determination described by one of the authors (38), which still exactly indicates values of 5 mgm. per cent, it is possible to reduce the quantities of inulin to the fifth part of those given by other authors.

During the first 10 minutes we infuse 60 cc. of 5 per cent inulin solution, then 4 cc. of 1 per cent inulin per minute continuously to the end. The rate of the infusion is regulated by a uniformly compressing tunnel clamp of 8 cm. in length. The quantity is measured by a burette communicating with the infusion flask. As the infusion flask is closed by a cock, the emptying time of the burette

TABLE I
Data on patient Skirn

Period	Urine flow	Plasma level			Clearance			Clearance ratio	
		Inulin	Creatinine	Urea	Inulin	Creatinine	Urea	Cr/I	U/I
I	cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	cc. per minute	cc. per minute	cc. per minute		
II	2.0		1.34	34.8		83.6	40.4		
III	2.0	26.1	1.34	33.5		84.4	40.8		
IV	2.7	26.1	1.34	33.5	90.1	92.7	51.0	1.03	0.56
V	2.11	25.8	1.34	33.5	74.4	78.8	42.2	1.06	0.51
	2.78	25.8	1.34	33.5	88.8	91.7	46.0	1.03	0.51

is measured with a stop watch. Twenty minutes after the beginning of the infusion of the 1 per cent solution, the plasma inulin has reached a constant value of 25 to 35 mgm. per cent. At this moment the first period begins. The urine and blood samples are drawn as described. A typical example of such an experiment is shown in Table I.

The results of the first 11 cases (Table II) show that the endogenous creatinine clearance corresponds very well to the inulin clearance

TABLE II
Inulin and endogenous creatinine clearances in normal subjects and in patients with slightly diminished renal function

Num ber	Sub- ject	Diagnosis	Urine flow	Clearance			Clear ance ratio Cr/I
				Inulin	Creat inine		
			cc. per minute	cc. per minute	cc. per minute		
1	St.	Normal	3.48	124	116.7		0.94
2	H.U.	Normal	1.80	139	159		1.14
3	H. Y.	Normal	0.75	107.8	108.5		1.00
			0.85	112.6	105		0.93
4	Ep	Normal	5.45	116	123		1.06
			4.85	116.8	117.6		1.00
			2.29	130	149		1.15
5	Sh. S.	Bronchial asthma	2.2	138.6	139		1.00
			3.5	151	159		1.05
			3.75	144.6	159.5		1.10
6	Shin	Hypertension	2.7	90.1	92.7		1.03
			2.11	74.4	78.8		1.06
			2.78	88.8	91.7		1.03
7	Ab	Pulmonary abscess	1.25	165.2	172.5		1.04
			1.0	160	172.2		1.08
			1.0	159	157		0.99
8	Al	Hypertension	5.32	71	79.5		1.12
			6.92	79.8	84		1.05
			10.85	96	98		1.02
9	Os.	Rheumatism normal	3.4	96.6	111.2		1.15
			7.8	113	132.8		1.17
10	Y. Y.		3.0	129.8	99.9		0.77
			2.16	105	83.7		0.80
			1.21	92.3	76.4		0.83
11	K. A.	Hypertension	7.2	116	85		0.73
			4.2	106	85.2		0.80
			5.0	108	95		0.88

Average = 1.03

TABLE III
Inulin and creatinine clearances in subjects with glomerulonephritis

Num ber	Sub- ject	Diagnosis	Urine flow	Clearance		Clear ance ratio Cr/I
				Inulin	Creat inine	
			cc. per minute	cc. per minute	cc. per minute	
1	Ke.	Chronic nephritis	0.90	5.1	8.5	1.67
			0.95	5.6	8.4	1.50
			0.87	4.9	7.9	1.61
2	Shab	Nephrosis	0.59	85.5	114	1.33
			0.75	80.6	106	1.32
			1.28	67.3	95.6	1.42
3	Shaz.	Chronic nephritis	1.22	4.9	5.6	1.14
4	Must	Acute nephritis	3.70	121.5	126	1.04
5	Ri	Chronic nephritis	3.72	118.5	126.5	1.07
			1.52	3.7	5.3	1.44
			0.75	2.0	3.45	1.73
			1.12	3.0	3.75	1.23
6	Is.	Acute nephritis	2.91	47.3	61.1	1.39
			3.20	53.4	67.8	1.27
			3.0	44.4	63.5	1.43

within the limits of error of the clearance determinations. On the average the creatinine/inulin ratio of the twenty seven separate periods is 1.03. This result justifies the opinion that the endogenous creatinine clearance, performed after Popper's method exactly indicates the filtration rate in normal persons or in patients with slightly diminished renal function (Cases 6 and 8).

So far, we have compared the excretion of these substances in only six as in the dog that diseases (Table. U) 15 per cent of urea is reabsorbed at extremely low concentrations of the neph and the urea clearance approaches the filtration rate. But as soon as the urine/plasma ratio of inulin has risen to 10, 35 to 40 per cent of the urea is reabsorbed. The reabsorbed share of the urea, however increases much more slowly

with increasing concentration of the urine. Therefore, in the range of the concentration ratio between 10 and 200, the urea reabsorption slowly increases from 40 to 70 per cent. So it seems that in this range other mechanisms influence urea absorption than those active at extreme dilutions.

Trying to explain this fact Smith (34) introduced the hypothesis of at least a double-sided water and urea reabsorption. During the "obligatory isoosmotic water reabsorption" up to 40 per cent of the urea is reabsorbed in the proximal segment of the tubule, while the urea absorption exceeding 40 per cent occurs in the distal tubule, together with the "facultative water reabsorption."

For purely diagnostic purposes and ignorant of these opinions, we compared the clearances of urea and endogenous creatinine in 111 cases. The results are arranged according to the concentration of the urine, expressed by the creatinine U/P ratio, in Table V. It is evident that each

TABLE V
Creatinine U/P ratio and urea reabsorption (111 cases)

Creatinine U/P ratio	Number of cases	Filtration rate	Urea reabsorption
		<i>cc per minute</i>	<i>per cent</i>
2- 10	8	16- 7	29
10- 20	11	11 -103	32
20- 40	14	25 -128	47
40- 60	19	21 -145	55
60- 80	11	38 -163	57
80-100	9	70 -140	62
100-120	13	46 -166	52
120-140	5	68 -128	66
140-160	7	55 -142	62
160-180	4	55 -163	54
180-200	4	17 -160	69
200-340	6	87 -142	75

column, except the first one, includes normal and pathological filtration values. The results agree very well with those of Chasis and Smith (5). In the extremely low concentration of pathological cases the urea reabsorption is on the average 29 per cent, in particular cases it is even as low as 10 per cent. When the diuresis of normal kidneys is great (column 2 and 3), the reabsorption amounts to about 40 per cent. With increasing concentration the reabsorption slowly increases until, at a creatinine U/P ratio of 40 to 180, it attains an average value of 57 per cent.

In high concentrations of more than 200, an average of 75 per cent of urea is absorbed, in particular cases even as high as 82 per cent. The conformity of these values with those of Chasis and Smith (5) can be considered as a further indirect proof of the fact that the endogenous creatinine clearance of Popper must approach the glomerular filtration.

As mentioned above, the results are to a certain extent contradictory to the idea of maximum and standard clearance, ϵ , at extremely low concentrations of urine. In clinical practice the glomerular filtration agrees rather well with the urea clearance, expressed as percentage of the normal value.

The results of the three comparisons are as follows:

1 The comparatively high conformity between the clearances of inulin and creatinine supports the hypothesis that the endogenous creatinine clearance expresses the filtration rate (Tables I and II). The obviously higher concentration of creatinine in some cases (Table III), however, is not in accordance with Shannon's laws of secretion in the tubules. Therefore, the problem of secretion of creatinine cannot yet be considered as definitely solved.

2 The fact that creatinine orally administered is more concentrated than endogenous creatinine renders the sole filtration of endogenous creatinine very probable (Table IV).

3 The conformity of the values for urea reabsorption with those found by Chasis and Smith further confirms the correctness of the determination of the filtration rate by endogenous creatinine.

These results do not prove with absolute evidence that the endogenous creatinine clearance and the filtration rate agree perfectly in all cases. For all clinical purposes, however, this evidence is sufficient and the determination of the filtration by the endogenous creatinine clearance is much easier and more convenient than by any other method.

THE DIAGNOSTIC VALUE OF PLASMA CREATININE AND ENDOGENOUS CREATININE CLEARANCE

In all the cases in which the size of the filtering surface of the kidney is reduced, be it as a con-

sequence of inflammation, by sclerosis or by any other kind of reduction of the vascular caliber the filtration rate will be reduced. So the filtration rate is a clear measure of the part of the kidney still in function. It is evident that the quantitative determination of the part of the kidney still functioning must be considered as the essential measurement of renal function. All other examinations of partial function must be of secondary importance. One must take into consideration of course, the possibility that some extrarenal factors may cause a temporary decrease of filtration. For instance, in cardiac decompensation the filtration may decrease to 20 cc., and a sudden loss of blood or a shock may depress the filtration almost to zero. Also in various conditions accompanied by hypochloremic

azotemia, caused by extrarenal factors and in certain cases of acute infection and jaundice, temporary reductions of filtration rate may often be found. Back pressure, caused by various urological conditions may also considerably reduce the filtration. All these conditions must be kept in mind when a reduced filtration rate is to be interpreted. Nor must it be forgotten that a normal filtration rate need not exclude pathological changes in the kidney.

1 Comparison between the endogenous creatinine clearance and the plasma creatinine

What are the laws for the excretion of an endogenous substance which is always supplied at a constant rate, and therefore shows a constant plasma level and which is only filtered but neither

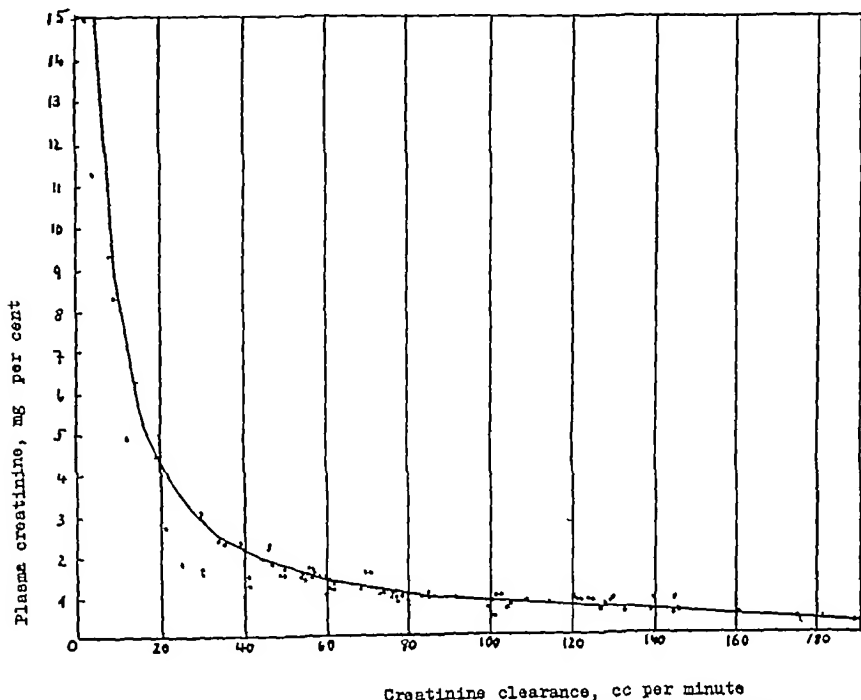


FIG. 1 RELATIONSHIP OF PLASMA CREATININE CONCENTRATION TO ENDOGENOUS CREATININE CLEARANCE (160 DETERMINATIONS)

reabsorbed nor secreted? Suppose that in a unit of time 100 cc of glomerular filtrate are formed at a plasma level of 1 mgm per cent and 1 mgm of the substance is excreted. If, the substance being supplied at a constant rate, the filtration decreases to 50 cc, the plasma or filtrate concentration must increase to 2 mgm per cent so that the excreted quantity remains the same. That means that the product of the plasma concentration and the filtration rate must be a constant value. If we could find out that this simple relation is valid for an endogenous substance, we shall have proved that this substance has been only excreted by filtration and neither reabsorbed nor secreted. Moreover, with the aid of this constant value we should be able to deduce the filtration rate only from the plasma value of this substance.

We have made an attempt to prove this relation for the endogenous creatinine by 160 determinations (Figure 1). Only adults of medium body surface have been examined. The average product of plasma creatinine and filtration rate has been found to be 86, *i e*, if the plasma contains 1 mgm per cent creatinine, an average of 86 cc of glomerular filtrate is formed. The average value of this product shows sufficient uniformity for different values of plasma creatinine (Table VI). For higher creatinine values (more than

TABLE VI

Product of plasma creatinine and filtration rate

Plasma creatinine mgm per cent	Number of determinations	Average product
0.4-1.0	60	89.8
1.0-1.5	46	89.7
1.5-2.0	27	85.0
2.0-3.0	11	81.4
3.0-4.0	3	84.2
4.0-5.0	5	83.7
5.0-6.0	3	93.3
6.0-15.0	9	50

6 mgm per cent) we obtained a lower average value in the 9 cases examined. For the moment we cannot draw theoretical conclusions from this small number of cases. Table VII demonstrates the distribution of the values of the product of plasma creatinine and creatinine clearance. It is evident that the product lies between 60 and 120 in 74 per cent of the 160 cases.

A serious objection to this conclusion has been

TABLE VII
Distribution of the product

Product	Percentage of determinations	Product	Percentage of determinations
30-40	2.4	90-100	18.1
40-50	6.2	100-110	10.0
50-60	6.9	110-120	6.2
60-70	13.8	120-130	2.5
70-80	14.4	130-140	3.1
80-90	11.5	140-150	3.1
		150-160	1.8

made by Van Slyke (42). The rate of creatinine formation per square meter of body surface is not the same in all subjects. Prolonged illness, with loss of muscular ability, and some other uncontrollable circumstances may reduce the creatinine output to less than one-half of the normal amount. Therefore, it is impossible to deduce the filtration rate from the plasma creatinine in every case. In the recent reviews on the excretion of creatinine (1, 41), no data concerning the reduction of the excretion of preformed creatinine are given. We therefore examined another series of 74 cases and found that in the majority of these the creatinine formation was comparatively constant. In 12 of 74 cases, *i e*, in 16 per cent, the formation was reduced one-half to three-fourths, in perfect accordance with the rates summarized in Table VII. These cases include cachexias, women with diabetes after a long fasting period, a woman with hypertension, and patients with kidney diseases in bad condition.

For the present, disregarding the fact that in 16 per cent of the cases the creatinine formation is reduced, we may conclude from the other cases that the endogenous creatinine is only excreted by filtration, as plasma creatinine and filtration rate are inversely proportional. The quantity of filtered and excreted creatinine remains constant within certain limits, independent of the plasma level. This fact is very important for clinical diagnosis.

As shown in Figure 1, at a reduction of the filtration rate below 80 cc the plasma creatinine is forced to increase to pathological values. This retention occurs at first in creatinine which in normal persons shows a very constant level, and which of all the components of the blood has the greatest U/P ratio. Filtration rate multiplied by plasma creatinine equals 86, 86 dl

plasma creatinine gives the filtration rate corrected for the normal body surface (173 sq.m.) So it is possible, with an accuracy sufficient for clinical purposes, to determine the rate of the glomerular filtration by the determination of one constituent of the blood and to obtain results which include the correction for the body surface. Holten and Rehberg (15) would have employed the endogenous creatinine for the determination of the filtration rate had they known an exact method for the determination of creatinine.

We estimate the filtration rate by deduction from the plasma creatinine chiefly in outside patients in whom the determination of the clearances would be difficult. In the hospital we continue estimating the creatinine clearance. After forming an opinion about the rate of creatinine formation in a patient by means of several preliminary tests and putting the obtained product into the calculation, it is possible to deduce the filtration rate for months solely from the plasma creatinine level

2 Plasma creatinine and urea

If the renal function, as measured by the urea clearance, has already decreased to 20 to 40 per cent of normal, the blood urea is still within the normal range of 50 mgm. per cent in more than half of the cases. These results demonstrate the uncertainty of the diagnostic value of normal blood urea contents in kidney diseases. In Figure 2 the relation of blood urea to creatinine is represented in 171 cases. In a great number of cases in which the urea content is found within the normal range of 50 mgm per cent, we can observe a distinct increase of plasma creatinine often to twice the normal value or more. As the two lines limiting the area indicate, no fixed relation exists between urea and creatinine. The content of urea in blood is greatly influenced by extrarenal factors. The level of creatinine in blood however, can indicate a considerable reduction of the filtration rate at a still normal urea value. Even Popper, Mandel, and Meyer's method (27) for the rapid estimation of renal deficiency, which is a qualitative determination of creatinine in blood performable within 3 minutes at the bedside, often indicates pathological results when the urea level of the blood is still normal. Popper and Brod (24) emphasize the superiority

of the simple determination of creatinine in plasma over the determination of the filtration. Physiological fluctuations of the filtration are of no importance in the estimation of the results.

It must be mentioned once more that the quantitative results represented here have only been found with Popper's method (26). The insufficiency of the original method must be the reason for the lack of conformity between the early investigators on the diagnostic value of plasma creatinine, and especially on the sequence of the retention of urea, non protein nitrogen, uric acid, creatinine and indican. (See discussion by Peters and Van Slyke (23)).

3 Filtration and urea clearance

As already quoted above filtration and urea clearance were compared in many cases. The findings of previous investigators (3, 13) have shown that in kidney diseases the decrease of the urea clearance expressed as percentage of normal was roughly parallel to that of the filtration. This is true chiefly for the field between 10 and 50 per cent of urea clearance, where both values nearly coincide.

In uremia with a filtration of less than 10 cc., the value of the urea clearance is sometimes a little higher. But that is of no practical importance. As has been stated this fact is to be explained by the extremely low capacity of concentration of these kidneys and by the reduced reabsorption combined with it.

We can often observe a divergence of urea clearance and filtration at a urea clearance of more than 70 per cent of normal. The filtration rate is normal but often not higher than the percentage of the urea clearance.

As a whole, we must state that the determination of the filtration rate by means of the endogenous creatinine and the urea clearance are of the same diagnostic value. Since the determination of plasma creatinine alone allows one to estimate the rate of the filtration, this simple method is sufficient for the diagnosis of the still functioning part of the kidneys. The only disadvantage of this method is the fact that 4 cc. plasma *i.e.*, 10 cc. blood are needed whereas an exact determination of urea can be made with 0.2 cc. blood.

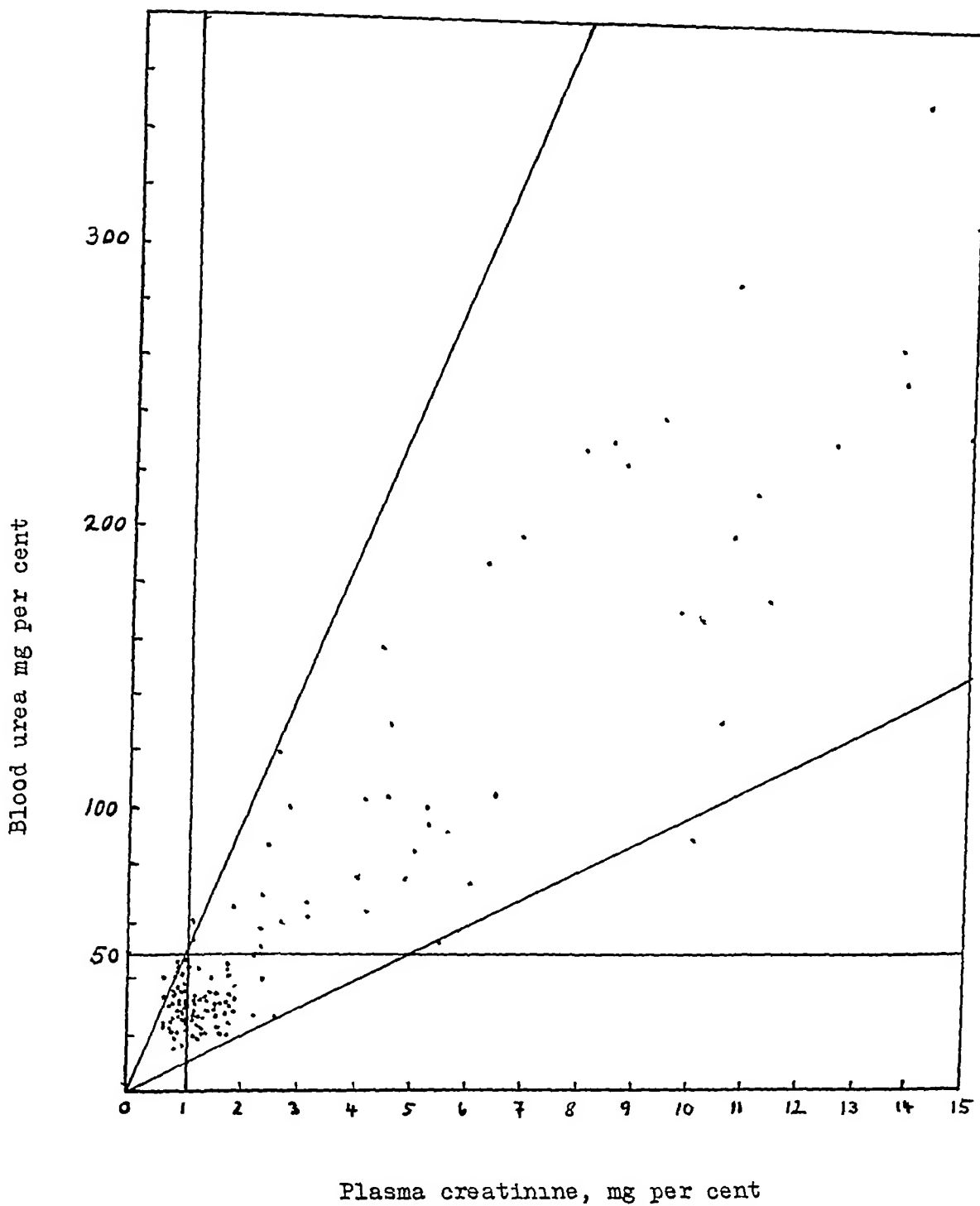


FIG 2 BLOOD UREA AND PLASMA CREATININE IN 171 CASES

The simple determination of plasma creatinine is a further step in the simplification of the diagnosis of renal function. It can in practice replace all determinations of glomerular function such as those of non protein nitrogen, urea urea clearance, the test of orally administered urea the administration of other substances and clearance determinations with other substances.

We call attention to the fact that the urine of urological patients with an indwelling catheter is often decomposed by bacterial action in the bladder and therefore the determination of urea in urine results in values which are too low and which give the illusion of too low urea clearances and of a more than normal urea reabsorption. In the cases of some of these patients we were of the erroneous opinion that we had to deal with an extreme urea reabsorption. In such cases only the creatinine clearance gives the right result.

4 Compensation and reabsorption uremia

Before we finish the discussion about the excretion of creatinine and urea we still have to deal with the ideas of "compensation" and "reabsorption uremia". Compensation means that, at a slightly reduced filtration together with an increased plasma creatinine, the retention of urea can be compensated by an actively reduced reabsorption. Thus a moderate reduction of filtration would not necessarily influence the actual urea excretion. In our patients we never observed this but, on the contrary, sometimes found a normal filtration rate and a slightly reduced urea clearance.

The term "reabsorption uremia" was used for the first time by Ferro-Luzzi (7) and was employed in a larger sense by Popper and Mandel (25). Yamaguchi (43) defines the action of the tubules not as active reabsorption but as a selective inhibition of the complete reabsorption. This faculty of selective inhibition of the reabsorption is supposed to be upset in serious deficiencies of the tubuli, so that creatinine and urea pass back into the blood in increased quantity. Contrary to Popper we believe that it is impossible to apply to cases typical of the course of glomerulonephritis these very interesting impressions which have been derived from experiments with toads. We are of the same opinion as Chasis and Smith (5)

who, by comparison of urea and inulin clearance, came to the conclusion that the idea of reabsorption uremia does not conform to the facts. The diffusion coefficient of urea is seven times greater than that of inulin (4). The normal walls of the tubules are impermeable to inulin and only a little permeable to urea. If we suppose that the permeability increases we ought to conclude that urea passes earlier and more rapidly than inulin, which means that the reabsorbed quantity of urea ought to increase. But the contrary is true. With decreasing capacity of concentration of the kidneys the reabsorbed quantity of urea decreases, as Chasis and Smith (5) and ourselves were able to demonstrate. This fact indicates a reduced and not an increased urea reabsorption. Until a final proof has been established the term "reabsorption uremia"—which would be suitable for the explanation of some facts otherwise still unexplainable—ought to be abandoned and any retention in cases typical of the course of glomerulonephritis should be explained only by reduction of the filtering surface.

In some other conditions however an exceptional behavior must be admitted. In the cases designated by McCance and Widdowson (17) as functional disorganization of the kidney, a temporary decrease of the filtration rate occurs by decrease of the blood pressure or by other extrarenal influences. In absence of any constant anatomical alteration these conditions are characterized by the functional integrity of the reabsorbing parts of the nephrons. In diabetic coma, severe salt deficiency (16) uncompensated alkalosis, dehydration, and certain other conditions the glomerular filtration decreases but the reabsorption on the part of the tubules is unstrained. The rise in blood urea may be accounted for by an additional urea reabsorption. Observations on chloride reabsorption may be explained in the same manner.

McCance and Widdowson (17) observed in diabetic coma an abnormal fall of the creatinine/inulin clearance ratio, but were not able to explain this fact by the existence of a reabsorptive mechanism. The observation of the low creatinine/inulin ratio may be erroneous by an analytical reason. The values of plasma creatinine given by McCance and Widdowson (17) are perhaps too high, as in diabetic coma great amounts of

acetoacetic acid and acetone are present in plasma. To demonstrate this assumption we selected period 1, patient 1, Table III from McCance and Widdowson (17). The inulin U/P ratio is 38, the creatinine U/P ratio 186 at a plasma creatinine level of 25.8 mgm per cent. If the creatinine U/P ratio were also 38, the plasma creatinine would be 12.6 mgm per cent. Regarding the great quantity of ketone bodies present in plasma during diabetic coma, we can suppose that the difference between the creatinine value found and the creatinine value deduced is in fact caused by ketone bodies.

METHODS

1 Determination of creatinine in plasma and urine according to Popper, Mandel, and Meyer (26)

In a test tube 4 cc. oxalated plasma are introduced with an Ostwald pipette into 12 cc. saturated picric acid, previously purified according to Benedict (2) and mixed by shaking vigorously. The mixture is immersed in boiling water for 15 seconds. The precipitate, after cooling, is resuspended by shaking and is filtered. To 10 cc. of the filtrate 0.5 cc. 10 per cent NaOH are added and mixed. The photometric reading is made after 20 minutes with a filter of 530 m μ and a depth of layer of 60 mm. As the mixture often tends to become slightly turbid, it is filtered once more 5 minutes before the reading. A blank analysis with 4 cc. water instead of plasma is treated in the same way and used for the compensation in the photometer. As the Beer-Lambert law is not strictly applicable, it is necessary to make an exact comparison curve. In concentrations of more than 4 to 5 mgm. per cent it is necessary to dilute the plasma twice, four, or even ten times.

The urine is diluted accordingly, usually fifty times and 4 cc. of the dilution are added to 12 cc. of the picric acid solution. Twenty minutes after the addition and mixing of 0.8 cc. 10 per cent NaOH, it is read in the photometer. If the urine contains albumin, it is filtered 5 minutes before reading. Absolutely clean tubes and pipettes are necessary to get reliable results.

2 Determination of inulin according to Steinitz (38)

After the desalbumination of 1 cc. oxalated plasma with zinc sulfate and sodium hydroxide, according to Somogyi (37), the quantitative Selivanoff reaction, modified by Roe (30), is performed. The principle of the method consists of the colorimetric determination of the levulose formed from the inulin during acid hydrolysis. The acid hydrolysis and the colorimetric reaction are performed simultaneously.

3 Microdetermination of urea according to Conway (6)

The principle and the technic of this method are described in another paper (39).

SUMMARY

Since the tubular secretion of exogenous creatinine has been demonstrated, the determination of the glomerular filtration by the administration of exogenous creatinine has become problematic. Since that time reliable methods for the determination of endogenous creatinine have been developed.

After a short discussion of Popper's method for the determination of creatinine, the results of 3 series of experiments, performed to show the suitability of this method for the determination of the glomerular filtration, are reported.

1 Comparison between the endogenous creatinine clearance and the inulin clearance

2 Comparison between the endogenous creatinine clearance and the exogenous creatinine clearance

3 Comparison between the endogenous creatinine clearance and the urea clearance

The results seem to prove that with Popper's method the glomerular filtration can be determined much more easily by the endogenous creatinine clearance than with any other method.

The determination of plasma creatinine is of high diagnostic value because it makes possible the estimation of the glomerular filtration rate by one simple blood analysis. This simple method can therefore replace any other test of glomerular function. It is a further step in simplifying the diagnosis of renal function.

The conception of reabsorption uremia is discussed, but cannot be maintained for the explanation of the retention of urea in cases of glomerulonephritis, except for the cases of functional disorganization of the kidney.

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STUDIES ON THE CONDITIONS OF GLUCOSE EXCRETION IN MAN

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The application of the filtration reabsorption theory to the explanation of glucose excretion has been of great importance for our knowledge of the factors involved in this process. In normal and pathologic conditions the quantity of glucose excreted is dependent only on three factors: the level of the capillary blood sugar, the filtration rate and the glucose threshold.

We may assume that the glucose level of the glomerular filtrate is the same as that of the arterial plasma. The quantity of the filtered glucose is determined by two factors: the level of the arterial blood sugar and the quantity of the filtration rate. Under normal circumstances the glucose is almost completely reabsorbed by the tubules. In a unit of time only a constant maximal quantity of glucose can be transported by the tubular cells, as Shannon and Fisher (14) demonstrated for the normal dog, Govaerts and Muller (3) for the diabetic dog. Under normal condi-

tions this reabsorbing capacity of the tubules is not fully utilized and the excretion of glucose is almost zero. When the blood sugar and in consequence the filtered quantity of glucose has been increased or has just risen to the full utilization of the reabsorbing capacity the blood sugar has reached the threshold. The quantity of the maximal reabsorbed glucose therefore depends on two factors: the level of the threshold and the quantity of the filtration rate.

If the blood sugar rises above this threshold, the maximal capacity of reabsorption is surpassed and the glucose appears in the urine. Glucose filtered — glucose reabsorbed = glucose excreted. This excess glucose which has not undergone reabsorption is now concentrated at the same rate as other substances which are neither reabsorbed nor secreted from the tubules, such as inulin or endogenous creatinine. One can express this relationship by the following equation (2)

$$\begin{aligned} (1) \quad & \frac{\text{creatinine excreted mgm. per minute}}{\text{plasma creatinine, mgm. per cent}} = \frac{\text{glucose excreted mgm. per minute}}{(\text{blood sugar} - \text{threshold}), \text{mgm. per cent}} \\ \text{or} \\ (2) \quad & \text{glucose threshold} = \text{blood sugar} - \frac{\text{urine sugar mgm. per cent} \times \text{plasma creatinine mgm. per cent}}{\text{creatinine in urine mgm. per cent}} \end{aligned}$$

So far the threshold in man has been determined by oral administration of glucose. The blood sugar is tested at close intervals and the appearance of sugar is noted in the urine. The disadvantage of this procedure lies in the fact that the rise of the blood sugar curve may be very rapid, and the peak very brief. Most of the authors have observed a high ascending and a low descending threshold. In this article we will not discuss whether this difference really exists or whether it is due only to the experimental conditions (cf. Govaerts and Muller (2), Peters and Van Slyke (9), Falia (1)). Moreover, it is only possible to provoke glycosuria by oral administration in a certain percentage of subjects (16).

To avoid these difficulties, we tried to fix the

threshold by another method that is as already stated by the determination of that part of the blood sugar which is treated like a substance that has neither been reabsorbed nor secreted. To render this possible, the existence of a frank glycosuria is indispensable. When glycosuria was absent it was produced by intravenous infusion of glucose in order to obtain a constant plasma level of glucose. For the following reasons it is impossible to compare the results of this method with the aforesaid oral administration method without certain considerations.

From their experiments with dogs Shannon and Fisher (14) have reached an equation according to which it is to be expected that small

concentrations of glucose will appear in the urine at normal plasma glucose levels, that there will be a little increase in the rate of excretion as the plasma level rises until the maximal rate of reabsorption is approached, and that the maximal rate of reabsorption will thereafter be rapidly attained" Govaerts and Muller (2, 3) obtained exactly the same results. They called that level of blood sugar at which the first traces of glucose appear "*seuil d'apparition*," according to the definition of C. Bernard, whereas they called the higher level of blood sugar, above which there exists a direct proportionality between "*supra-limitary*" blood sugar and the amount of glucose excreted, the "*seuil maximum*"

With our method we have not determined the threshold of appearance, but the maximum threshold. It is rather probable, however, that these two thresholds are not very different from each other. Experiments in dogs show "that a small but significant amount of glucose first appears in the urine when the plasma concentration is 10 to 20 mgm per cent below the level at which the maximal rate of reabsorption is reached." One of our experiments seems to demonstrate that this difference is not greater in man, and that between the level of the threshold of appearance and that of the maximum threshold only very small amounts of glucose are excreted. These amounts are not important for the excretion rate of glucose in diabetic patients.

METHODS

In patients with a spontaneous glycosuria technic A was employed. Urine and blood were collected as described by Möller, McIntosh and Van Slyke (6) for the urea clearance. At the beginning, in the middle, and at the end of the test, the capillary blood sugar was determined by the method of Hagedorn and Jensen. The results of these determinations must not differ very much from each other. The sugar in urine was determined by polariscopic examination after previous precipitation with lead acetate. The creatinine in blood plasma and urine was determined by the method of Popper and Mandel (10). Patients with acetonuria were excluded from the test, as the values of creatinine in plasma and urine increase considerably in the presence of acetone. An attempt was made to remove the acetoacetic acid and the acetone by boiling the picric acid filtrate, but the results were not satisfactory since, in spite of the removal of acetone, the chromogenic substance increased slightly, even during a short boiling period.

Glucose was introduced in patients without glycosuria by an intravenous infusion, and the period was started if a frank glycosuria was present (technic B). In some cases the inulin clearance was determined simultaneously (technic C). The collection of urine and blood, the technic of infusion and the analytical methods were the same as described in the previous paper (15). The amount of glucose necessary to produce a glycosuria was approximately the same as described by Sansum and Woodyatt (13). Glucose was given as a 20 per cent solution at a rate of 5 cc. per minute. After 25 to 30 minutes glucose appeared in the urine, and the first period began.

Increased plasma glucose gives a faint color reaction with the modified Selivanoff's reagent (11). According to the increase of blood glucose during the experiment, glucose was added to the fasting blood filtrate in order to compensate for this color. The value of extinction of this blank analysis was subtracted from the other values. Glucose in blood and urine was determined as already described. Only in experiments with inulin was the sugar in urine determined by the reduction of titrated Benedict's reagent.

Normal subjects

Table I shows the results in 4 normal subjects. The maximum threshold in these subjects lies between 200 and 280 mgm per cent. In Case 4 we tried to determine the difference between the threshold of appearance and the maximum threshold. After a priming infusion of 10 per cent glucose with 5 per cent inulin, a second infusion of 10 per cent glucose with 1 per cent inulin was given at a rate of 5 cc per minute until the blood sugar remained constant between 173 and 179 mgm per cent. At the same time the filtration rate was determined. Then a third infusion of 20 per cent glucose with 1 per cent inulin was given at the same rate. The first glucose could be found in the urine at a blood sugar level between 194 and 209 mgm per cent. At a blood sugar level of 220 mgm per cent the same value for the threshold was found. Unfortunately, the patient was not able to bear the infusion any longer but it seems probable that the threshold would not have increased much at higher blood sugar levels.

Diabetes renalis

We were recently able to examine 4 cases of true renal glycosuria. As these cases are relatively rare, a short report will be of interest. The results of these cases are given in Table II.

TABLE I
Normal subjects

Technic	Subject	Age	Urine flow	Creatinine U/P ratio	Sugar in urine	Supra-renal blood glucose	Blood sugar	Threshold	Reabsorbed glucose
			cc. per minute		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per minute
B	Kasim	18	11.9	10	1100	110	389	279	332
			16.4	8.5	800	94	376	282	392
			18.5	7.75	700	90.5	334	244	348
			14.0	8.95	300	33.5	305	272	339
B	Ismail	45	16.4	10	700	70	279	209	343
			12.4	10	800	80	299	219	271
			12.0	10	900	90	309	219	264
			3.85	25.5	1280	51.5	298	247	236
C	Shemsi	36	6.28	17.5	1310	76	323	247	268
C	Hikmet	25	16.8	7.65	45	5.9	201	195	251
			12.1	7.65	210	27.4	220	193	180

Case 1 Münevver is a female of 27 years. Towards the end of the first pregnancy sugar was found in the urine during a casual examination. At a second examination some weeks after the delivery sugar was found again and assumed to be lactose. Three months after the delivery the patient was admitted to the hospital. The daily sugar output fluctuated between 35 and 50 grams the fasting blood sugar level being 88 mgm. per cent. The sugar was identified as glucose as follows:

1 Reduction and polariscopic examination gave the same quantity of glucose.

2 The fermentation with yeast began quickly. After complete fermentation the capacity of reduction disappeared.

3 The tests of Rubner, Wohlg and Malfatti for lactose were negative (8).

4 The characteristic formation of mucic acid was negative.

5. The phenyllosazone was formed quickly and copiously

during heating. Lactosazone, however, is soluble in the heat.

The results of tests 2 to 5 proved the absence of lactose.

The oral administration of 50 grams of glucose was followed by a quite normal blood sugar curve. The sugar in urine rose to 6.3 per cent. After the injection of 12 units of insulin the blood sugar decreased during 3 hours from 109 to 69 mgm. per cent. At this level the glucose excretion ceased.

During the time of hospitalization the daily excretion of sugar was 30 to 50 grams at an intake of 250 grams of carbohydrates. The fasting blood sugar level was always normal. During the observation time, from April to December 1933, no change occurred.

Case 2. Shevket is a male of 30 years. Sugar was found in the urine during a casual examination. The glucose tolerance test had been performed in another hospital and had been followed by a normal blood sugar

TABLE II
Diabetes renalis

Date	Technic	Subject	Age	Urine flow	Creatinine U/P ratio	Sugar in urine	Supra-renal blood glucose	Blood sugar	Threshold	Reabsorbed glucose
				cc. per minute		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per minute
April 21 1938	A	Münevver	27	0.57	207	3850	18.6	88	69	82
Feb 17 1939	A	Shevket	30	0.51	240	600	2.5	85	82	102
April 20, 1939	A	Agop	18	0.53	286	1000	3.5	98	94	143
June 2 1939	A	Ayshe	37	0.45	131	140	1.0	53	52	31
June 6 1939	A	Ayshe	37	0.56	234	2500	10.7	91	80	105
				0.397	307	3400	11.0	91	80	98
June 13, 1939	A	Ayshe	37	1.02	137	2000	14.6	88	73	103.5
				0.74	137	2500	18.3	97	79	80
				0.50	132	3400	25.7	107	81	54
				0.59	125	5400	43.2	116	73	53
				0.84	101	4700	46.5	109	63	53
				1.03	104	3000	28.9	89	60	65
				0.54	128	2400	18.7	86	67	46.5
				0.88	157	3000	19.1	87	68	94
				0.74	157	3500	19.1	87	68	79.5

curve, with abundant sugar excretion in the urine. We had no occasion to study this case more completely.

Case 3 Agop is a boy of 17 years. The first examination took place on April 20, 1935. Some time before this date a glycosuria was found incidentally and the existence of a diabetes mellitus was assumed. The result of the glucose tolerance test at that time was normal, but the sugar excretion rose to 3 per cent. The tolerance test with 100 grams of white bread gave a similar result. Since then we saw the patient only at irregular intervals. The daily glucose output was always about 20 grams, the fasting blood sugar being normal. The determination of the threshold was made on April 20, 1939.

Case 4 Ayshe is a female of 37 years. Four months ago she had a delivery with forceps. Twenty-five days later a manifestation of syphilis was found in her mouth. The Wassermann test was positive. Treatment with neosalvarsan and bismuth was begun. During the treatment sugar was found in her urine for the first time. The doctor assumed a diabetes mellitus, but dietetic treatment was without success. She was hospitalized on May 31, 1939. The sugar in the urine was identified as glucose as described in Case 1. As the resident physician had at first believed the case to be a diabetes mellitus, a fasting day and afterwards a vegetable day were ordered. On the following day, June 2, the threshold of glucose was found at 52 mgm. per cent at a glycosuria of 0.14 per cent. Now the patient was given usual food which contained more than 250 grams of carbohydrates. The daily sugar excretion rose to 25 to 40 grams. The glucose tolerance test on June 5 showed only a slight hyperglycemic response. The blood sugar rose from 77 mgm. per cent to 119 mgm. per cent, at a sugar excretion up to 6.8 per cent. The determination of the threshold was repeated on June 6, and 80 mgm. per cent were found at a sugar excretion of 3.5 per cent. On June 8, 10 units of insulin were injected into the fasting patient. The blood sugar fell from 71 mgm. per cent to 33 mgm. per cent. Even at this low blood sugar level in urine, 0.2 per cent sugar was excreted. After a week the determination of the threshold was made once more and repeated each hour from morning to afternoon. The patient is still in the hospital. Every day she excretes 20 to 35 grams of sugar at a normal blood sugar level.

In all the cases there exists an obvious inability of the tubules to reabsorb glucose in sufficient quantities. No conclusions concerning a uniform etiology can be drawn from these observations. The anamnesis gave no evidence of hereditary factors. The rôle of pregnancy in Cases 1 and 4 is not clear, as the non-existence of glycosuria before pregnancy has not been proved.

Monasterio (7) was the first to detect an anatomical anomaly of the renal tubules in one case of renal glycosuria. An enormous dilatation of the

lumen with flattening of the tubular epithelium was found. In our cases we had no occasion to suggest an operation to the otherwise normal patient. If further evidence from more cases is added, it will perhaps be possible to characterize some of the cases of renal glycosuria as an anatomical anomaly of the kidney.

We will not discuss here the mechanism of the failure of the tubular cells to transfer glucose. The results of Ruhl and Thaddea (12), who treated 2 cases with adrenal cortex hormone, are not yet very conclusive. Hoff (4) demonstrated the influence of lactoflavine and corticosterone on the phlorizin glycosuria.

The values of the threshold, determined during insulin hypoglycemia as well as by the "supra-limitary" blood glucose, agree very well in Case 1. In this case a difference between the threshold of appearance and the maximum threshold does not seem to exist. In Case 4, however, this difference seems to be obvious. After 2 fasting days the threshold is 52 mgm. per cent at a just perceptible glycosuria. During insulin hypoglycemia the threshold appears to lie much lower still. A complete renal diabetes seems to exist because the greatest part of the blood sugar value of 33 mgm. per cent, determined by the method of Hagedorn-Jensen, is formed by non-glucose-reducing substances. If glucose is filtered more copiously, however, the threshold rises to 70 to 80 mgm. per cent.

Diabetes mellitus

The determination of the threshold in patients with diabetes mellitus gave the same values as in normal subjects (Table III). The tests of some patients of advanced age and of patients whose diabetes had existed for a very long time, however, sometimes gave much higher values. In clinical practice this fact has been known for a long time but until now an explanation for the increased capacity to reabsorb glucose has not been found.

Reduced kidney function

One patient with amyloidosis in the terminal stage and with extremely low kidney function, and 1 patient with malignant hypertension were examined with technic B (Table IV). The de-

TABLE III
Diabetes mellitus

Date	Technic	Subject, age and sex	Urine flow	Creatinine U/P ratio	Sugar in urine	Supra- limitary blood glucose	Blood sugar	Thresh- old	Reabsorbed glucose
			cc. per minute		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per minute
May 2 1938	A	Veysel m. 24	1.09	51.2	1400	27.3	237	210	118
June 28 1938	A	Pardow f. 67	0.58	148	1550	10.5	205	195	160
Mar 13 1939	A	Shahs f. 39	0.62	174	8500	49	304	255	326
April 25 1939	A	Hanife f. 50	1.10	118	600	5	272	267	346
May 15 1939	A	Baha m. 36	3.75	44.4	5000	113.5	399	286	475
			3.87	43.2	5000	111	403	292	487
May 22, 1939	A	Baha m. 36	1.13	98	2940	30	295	265	294
			1.41	74.5	3120	42	303	261	269
			1.87	57.7	3850	67	312	245	270
			1.85	57.7	4270	74.3	324	250	271
May 13 1939	A	Psaliti f. 38	0.48	364	1700	4.7	260	255	444
June 7 1939	A	Mehmet m. 26	0.55	225	4670	20.8	280	259	321
			0.55	225	4670	20.8	259	238	295

Diabetes mellitus with high glucose threshold

June 21 1939	B	Ziya m. 62	4.18	22.5	3060	136	506	370	348
			4.50	19.2	3900	203	570	367	317
			5.42	15.4	3880	252	620	368	308
June 26 1939	B	Ahmet m. 65	3.30	31.6	3870	122.5	447	325	338
			5.73	30.5	3560	116.5	472	356	622
			5.39	26	3620	139.5	496	357	500
June 28 1939	B	Özat m. 63	3.29	32.4	2100	65	417	352	374
July 7 1939	C	Mahir m. 60	3.323	18.5	1190	64.3	372	308	184
			4.28	18.2	2030	112	429	317	247
			5.00	17.1	2260	132	457	325	278
			6.33	13.5	2050	152	470	318	272
July 12 1939	A	Zehra f. 44	0.98	64.7	900	13.9	307	293	186
			1.51	60.2	1000	16.1	312	296	269

creased excretion of glucose in patients with reduced kidney function depends only on the reduction of the filtration rate. The filtered quantity of glucose decreases so much that the reduction of the filtering surface causes a retention of sugar. The reabsorbed quantity decreases in the same proportion. The glucose threshold remains absolutely unchanged within the same limits as in the normal subject. The only explanation for this fact is that a reduction of the filtration at

glomerular lesions is accompanied by a corresponding reduction of whole functional units of nephrons. In Case 1 the filtration decreased to a minimal amount whereas the capacity of glucose reabsorption in the still functioning nephrons remained normal. Suppose that in a normal subject the quantity of reabsorbed glucose is 300 mgm. per minute, at a number of 2,000,000 nephrons then one of them reabsorbs 0.15 microgram. In our case only 3.6 mgm. glucose could

TABLE IV
Reduced kidney function

Technic	Subject and sex	Diagnosis	Urine flow	Creatinine U/P ratio	Filtration rate	Sugar in urine	Supra- limitary blood glucose	Blood sugar	Thresh- old	Reabsorbed glucose
			cc. per minute		cc. per minute	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per minute
B	Soy m.	Amyloidosis	0.84	2.16	1.82	580	269	471	202	3.6
			0.89	2.06	1.83	715	347	545	198	3.7
B	Eman m.	Chronic nephritis	1.0	24.5	24.5	270	11	272	261	64
			1.44	17.95	25.9	660	37	298	261	67.7
			1.81	13.75	24.9	870	63	336	273	66

TABLE V

Interaction of blood sugar level, filtration rate and renal threshold during excretion of glucose under various conditions

Condition	Glucose filtered		Glucose reabsorbed		Glucose excreted at
	Blood sugar level	Filtration rate	Renal threshold		
1 Normal	Normal	Normal	Normal		Blood sugar values artificially increased above the normal threshold
2 Phlorizin glycosuria	Normal	Normal or slightly reduced	Low or non-existent		Normal or decreased blood sugar values
3 Diabetes renalis	Normal	Normal	Low		Normal or decreased blood sugar values
4 Pregnancy glycosuria	Normal	Normal	Low		Normal or decreased blood sugar values
5 Diabetes mellitus	Increased	Normal	Normal		Blood sugar values increased above the normal threshold
6 Diabetes mellitus in advanced age	Increased	Normal	Increased		Relatively high blood sugar values
7 Reduced renal function	Normal	Reduced by destruction of functional units	Normal		Very high blood sugar values after injection of glucose
8 Diabetes mellitus with reduced renal function	Increased	Reduced by destruction of functional units	Normal or increased		Very high blood sugar values
9 Functional disorganization of the kidney	Normal	Reduced with preserved tubular function	Extremely increased		Extremely high blood sugar values after injection of glucose
10 Diabetic coma with functional disorganization of the kidney	Increased	Reduced with preserved tubular function	Extremely increased		Extremely high blood sugar values

be reabsorbed per minute. If we assume that in this case only 2 to 3 per cent of still functioning nephrons exist, that is 40,000 to 60,000, the quantity reabsorbed from one nephron is nearly the same as in the normal one.

In cases with reduced kidney function caused by decrease of the number of functional units, glucose is retained like other constituents of the plasma. Only at very high blood sugar levels sufficient glucose is filtered to cause a frank glycosuria. We did not examine cases of diabetes with kidney disease, but it is beyond any doubt that the low glycosuria of these patients has to be explained in the same manner. In some patients who have suffered from diabetes for a long time, the glucose threshold is very high. If this condition is complicated by a kidney disease, a very high blood sugar will be necessary to produce a glycosuria.

Functional disorganization of the kidney

In certain conditions the filtration decreases temporarily by decrease of the blood pressure or by other extrarenal influences without any lesion of the single nephron itself. McCance and Widowson (5) call this condition the "functional disorganization of the kidney." The filtration rate in the single glomerulus decreases, but the function of those parts of the tubules where glucose is reabsorbed remains unaltered. The filtered quantity of glucose decreases, while the reabsorbed quantity remains constant, and the excretion of glucose is greatly reduced or ceases completely. Shannon and Fisher (14) have demonstrated this process by an instructive experiment in Table III of their paper. In a decerebrated dog the reabsorption of glucose is examined before, during and after the application of a clamp

to the upper abdominal aorta. During artificial decrease of the blood pressure to 50 to 66 mm Hg the maximal reabsorbed quantity of glucose remains constant in spite of the reduction of the filtration rate and corresponding reduction of the filtered quantity of glucose by half. The glucose threshold, which before tightening the clamp lay between 360 and 370 mgm. per cent, suddenly rises to 620 to 650 mgm. per cent.

In the condition of functional disorganization of the kidney two factors retard the appearance of glucose in the urine. The first one is the retention of glucose caused by the decrease of the filtration, the second one is the unaltered reabsorbing function of the tubules, by which they are able to reabsorb glucose from the decreased filtrate with an extremely high threshold value. This condition may be called reabsorption hyperglycemia.

It has been known for a long time that often in diabetic coma, in spite of extremely high blood sugar values only traces of sugar are found in the urine. Sometimes, in spite of the presence of all the other symptoms, the diagnosis may be doubtful for the doctor because of the faint or negative reaction of sugar in the urine. McCance and Widdowson (5) have demonstrated that in such cases glucose is reabsorbed at a normal rate from a reduced glomerular filtrate.

The interaction of the three factors on which depends the excretion rate of glucose under various conditions is summarized in Table V.

SUMMARY

The appearance of glucose in urine depends on three factors: the blood sugar level, the filtration rate, and the renal threshold of glucose.

The tubular reabsorption of glucose has been examined by simultaneous determination of the endogenous creatinine clearance or the inulin clearance, the glucose excretion and the blood sugar level in 4 normal subjects, in 4 cases of renal diabetes, in 12 cases of diabetes mellitus, and in 2 cases of kidney disease.

The results of these various conditions have been discussed.

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ANTIBODY FORMATION IN CASES OF LOBAR PNEUMONIA TREATED WITH SULFAPYRIDINE

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For many years it has been known (1, 2, 3, 4) that at the time of the crisis in lobar pneumonia, or immediately thereafter, an excess of type-specific antibody can be demonstrated in the blood serum of the patient. While this phenomenon is not absolutely invariable, most observers have agreed that it occurs in the great majority of instances. Conversely, in cases going on to a fatal termination where crisis does not occur no type specific antibody can be demonstrated in the patient's serum during the course of the disease. These facts have led to the belief that spontaneous recovery in lobar pneumonia is intimately related to the patient's capacity for producing type specific antibody in such amount as to react with all the available antigen in the body and actually to appear in excess in the blood serum. Moreover, in recent years the phenomenon has been used as a quantitative guide to serum therapy, administration of therapeutic serum being continued until an excess of antibody could be demonstrated in the patient's serum.

Since the introduction of sulfanilamide and related compounds in the treatment of infections there has arisen intense speculation as to their mode of action. In view of the fact that these drugs while definitely bacteriostatic are not bactericidal it is obvious that the ordinary defenses of the body must play an essential role in the successful operation of the drug and evidence has been accumulated to show that phagocytosis of bacteria is more effective after they have been exposed to the action of sulfanilamide. In addition, the role of humeral antibodies has been studied experimentally, and it is the conclusion of a number of investigators that the effectiveness of these drugs is considerably enhanced if they act in the presence of immune serum.

In the case of human infections treated with sulfanilamide, an appraisal of the importance of active or passive immunity has not yet been made

and as this is a subject of some practical significance it was considered worth while to investigate it further. The opportunity to do so was afforded by the introduction of sulfapyridine. Since this drug is an effective therapeutic agent against all types of pneumococcus it was possible to study the immune reaction in human lobar pneumonia a disease in which the development of active immunity was known to be of critical importance. Therefore, beginning in February 1939 each case of lobar pneumonia treated with sulfapyridine at the Presbyterian Hospital was investigated for the appearance of type specific antibody.

Of the principal immune bodies which have been investigated in connection with lobar pneumonia, i.e. protective substances agglutinins, and precipitins we elected to study the latter. The precipitin reaction with specific soluble substance of pneumococcus has been the subject of extensive investigation by Heidelberger and his co-workers. That the type-specific precipitin and the agglutinin are the same antibody now seems to have been definitely proven (5). Moreover, an exceedingly close correlation has been shown to exist (6) between this antibody and the mouse protecting power of immune serum. As Heidelberger and Kendall (7) have stated while other reactions are more delicate, the precipitin test is among the most specific and least subject to errors and technical difficulties. The appearance of precipitins in the blood serum of thirteen patients recovering from lobar pneumonia eight of whom had received serum therapy, was found by Tillett and Francis (8) to coincide with the appearance of agglutinins. For these reasons the precipitin test with type-specific polysaccharide was used in this study according to the same technique which has been used in this clinic as a routine method of determining the adequacy of serum therapy.

TABLE I
Type-specific antibodies investigated in nineteen cases of sulfapyridine-treated pneumonia

Case number	Sex	Age	Duration of disease before treatment	Type	Lobe	White blood cells	Temperature before treatment	Date treatment started	Date temperature normal	* Precipitin tests	Total dose sulfapyridine grams	Remarks
1	F	58	3 days	iii	RUL	18,040 P 85%	104	February 1	February 1	February 7 00 February 14 0+ February 15 ++ February 17 00 February 20 00	24	
2	F	28	2-3 days	i	RLL	20,500 P 91%	105	February 5	February 7	February 7 00 February 8 00 February 9 00 February 10 00 February 11 00 February 13 00 February 14 00 February 15 00 February 16 00 February 17 00 February 18 00 February 20 00 February 23 00	49	
3	M	49	2 days	vii	RUL	18,550 P 94%	104	February 11	February 12	February 14 00 February 15 00 February 16 00 February 17 00 February 20 00 February 21 00 February 25 00	22	Past history of paroxysmal hemoglobinuria Blood and spinal fluid Wassermann positive
4	M	51	5 days	iv	LLL	15,680 P 85%	104	February 28	March 1	March 1 00 March 2 00 March 3 00 March 4 00 March 6 00 March 8 00 March 10 00	22	Rheumatic heart disease Auricular fibrillation Not decompensated
5	M	59	3-4 days †	i	LLL	14,160 P 91%	104	February 20	February 23	February 21 00 February 23 00 February 25 00 February 27 00 February 28 00 March 3 00 March 7 00 March 9 00 March 14 00	33	

* First column = two hour reading

Second column = overnight reading

TABLE 1—Continued

Case number	Sex	Age	Duration disease before treatment	Type	Lobe	White blood cells	Temperature before treatment	Date treatment started	Date temperature normal	* Precipitation tests	Total dose, sulfapyridine, grams	Remarks
6	F	35	3 days	xiv	RUL	21,680 P 92%	104	March 2	March 5	March 4 00 March 7 00 March 8 00 March 9 00 March 10 00 March 15 00 March 17 00 March 20 00	32	
7	M	60	3 days	iii	LLL	8,040 P 80%	104	March 4	March 7	March 6 00 March 7 00 March 8 00 March 9 00 March 11 00 March 14 00 March 17 00 March 20 00 March 21 00 March 24 00 March 28 00 March 31 00 April 3 00	54	Sulfapyridine stopped on seventh day of treatment. Next day temperature rose to 102. Drug once more started temperature promptly fell
8	M	23	8 hours	i	RLL	37,100 P 93%	104	March 8	March 10	March 10 00 March 11 00 March 14 00 March 17 00 March 20 00	32	
9	M	50	6 days	iii	LUL	14,000 P 94%	102.5	March 10	March 11	March 11 00 March 14 00 March 16 00 March 20 00 March 21 00 March 24 00 March 28 00	30	
10	F	29	6 days	v	RLL	21,000 P 93%	103	March 14	March 15	March 16 00 March 17 00 March 20 00 March 21 00 March 24 00 April 6 00	36.5	Received Type XIX antipneumococcus serum before admission. On March 19th developed serum disease, acric pleurisy and a rash thought to be due to sulfapyridine
11	M	51	2 days	vii	LLL	30,480 P 90%	104	March 20	March 21	March 21 00 March 24 00 March 27 00 March 28 00 March 31 00 April 3 00	32.5	

TABLE I—Continued

Case number	Sex	Age	Duration of disease before treatment	Type	Lobe	White blood cells	Temperature before treatment	Date treatment started	Date temperature normal	* Precipitin tests	Total dose sulfapyridine grams	Remarks
12	M	31	1 day	viii	LLL	24,000 P 88%	102	April 3	April 5	April 5 00 April 6 00 April 7 00 April 9 00 April 10 00 April 11 00 April 13 00	29	
13	M	36	2½ days	vi	LLL	12,400 P 91%	104	April 7	April 8	April 8 00 April 10 00 April 11 00 April 14 ++ April 15 ++	22.5	
14	M	39	4 days	iii	RLL LLL	10,760 P 92%	102.5	April 13	April 14	April 14 00 April 17 00 April 19 00 April 20 00 April 22 00	40	
15	M	57	3 days	i	RLL	22,200 P 88%	103.5	April 12	April 14	April 17 00 April 19 00 April 21 00 April 24 ++ April 25 ++ April 26 ++ April 27 00	52	Blood culture positive Precipitin reaction most strongly positive on April 24, diminished in intensity the next two days
16	F	59	5 days	vii	RLL	22,120 P 85%	104	April 11	April 13	April 18 00 April 20 00 April 25 00 April 27 00	32	
17	M	18	3 days	xviii	LLL	31,200 P 94%	103.5	April 15	April 16	April 17 00 April 20 00 April 21 00 April 27 00	23	Had hematuria during convalescence attributed to sulfapyridine
18	M	71	4 days	iii	RLL	20,600 P 90%	104	April 14	April 16	April 17 00 April 19 00 April 21 00 April 22 00 April 24 00 April 28 00	28.5	Had hypertensive cardiovascular disease Began to fibrillate on day after admission
19	F	22	2 days	i	RLL	29,600 P 85	106	April 12	April 15	April 21 00 April 23 ++ April 25 ++	40	

MATERIALS AND METHODS

Serum was obtained from each case of lobar pneumonia as often as was practicable during the entire hospital stay. Detection of antibody was made by means of the precipitin reaction with type-specific polysaccharide according to the technique of Dr Michael Heidelberger. To one cc. of serum was added one drop of a 1:50,000 dilution of specific polysaccharide¹ derived from the same type of pneumococcus as that isolated from the patient's sputum. At the end of an hour if the test were negative, three or four more drops were added. The tests were read after two hours at room temperature, and again after a night at ice box temperature. (Except in one instance, all the positive reactions were clear-cut at the end of two hours.) As a control another tube was set up with a few drops of normal salt solution. In addition, all human serums were set up with type-specific anti-serum to detect the presence of circulating polysaccharide. (This reaction was only found to be positive in one case as therapeutic serum was promptly administered, this case is not included below.) The results of the tests for type-specific antibody are given in Table I.

RESULTS

Nineteen cases of lobar pneumonia treated with sulfapyridine were investigated for the appearance of type-specific antibodies. Altogether one hundred and twenty seven samples of serum were tested by means of the precipitin reaction with specific polysaccharide. Of the nineteen cases only four were shown to have an excess of type-specific antibody in the blood and in these four the antibody did not appear at the time of or immediately after the 'crisis' induced by sulfapyridine. As nearly as can be determined from the data, antibodies in these four cases were first detectable after about a week of normal temperature. A study of the fever curves in the whole series indicates that the temperature fell to normal following sulfapyridine therapy within twenty four hours in nine cases within forty-eight hours in six, and within seventy two in four cases. The fall to normal in those cases developing antibodies occurred in twenty four hours in two cases and in forty-eight and seventy two hours in the other two respectively—a result exactly comparable to the rest of the series. Moreover the duration of disease before sulfapyridine therapy was begun was no greater in the cases which developed antibodies than in those which did not.

¹ The specific polysaccharides used in these tests were kindly supplied by Dr Michael Heidelberger to whom thanks are due, not only for them, but for invaluable advice.

Seven other cases were studied but not included in the series listed above, because each received, in addition to the sulfapyridine, a certain amount of therapeutic antipneumococcus serum. Two of these were due to Type I pneumococcus and both promptly showed an excess of antibody after serum therapy five were due to Type III, and, of these, three promptly showed an excess of antibody.

DISCUSSION

These observations indicate that in nearly eighty per cent of a small series of cases of lobar pneumonia treated with sulfapyridine recovery took place without the appearance of an excess of type-specific antibodies. In view of the fact that the production of an excess of antibodies has hitherto been regarded as an essential part of the mechanism of spontaneous recovery from pneumonia, it must be concluded that sulfapyridine has supplanted at least to some degree this part of the immune mechanism. There is no evidence of course, that antibody production ceases to occur in the presence of sulfapyridine. On the contrary, it is highly probable that it occurs at least to some extent. Such indeed, is suggested by the fact that in the cases where antibody appeared in excess, it did so later than has been observed in untreated cases implying that production has been going on at a slower rate, probably because the stimulus to antibody formation is lessened through the action of the drug on the invading organism. Moreover, this abnormally low rate of antibody formation may explain the well known clinical finding that if treatment with sulfapyridine is stopped too soon even if the patient has had no fever for several days a recrudescence of the pneumonia may take place. Finally, these observations do not settle the question as to whether therapeutic antiserum should or should not be given along with the drug, they merely indicate that demonstrable antibodies are not essential to recovery.

SUMMARY AND CONCLUSIONS

1 Nineteen cases of lobar pneumonia treated with sulfapyridine were studied for the appearance of type-specific antibodies in the blood serum, as judged by the precipitin reaction with specific polysaccharide.

2 In only four instances was an excess of antibodies demonstrated, and in these cases antibodies were not noted until after the patient's temperature had been normal about a week

3 The significance of these findings is discussed

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ELECTROPHORETIC ANALYSIS OF PLASMA AND URINARY PROTEINS

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Accurate analysis of the protein fractions of human plasma has in the past been very difficult and time-consuming. Fractional precipitation by neutral salt, as in Howe's method (1) has proved useful because of its simplicity and because the most important pathologic variations in plasma proteins involve a gross diminution in the albumin fraction. Such methods have definite limitations. Butler's careful study (2) of curves describing the 'salting-out' of human and horse serum demonstrated that the precipitation ranges of the globulin fractions overlapped one another grossly and that even the albumin-globulin separation was by no means sharp. That the albumin fraction after a single precipitation with neutral salt is grossly contaminated by globulin becomes most obvious when sera from nephrotic patients are studied. On dialysis of this 'albumin' fraction against running distilled water a large amount of the protein separates as a water insoluble precipitate.

The simple salting-out methods are thus very inaccurate in the cases in which they are of most interest. Combinations of dialysis isoelectric precipitation and neutral salt precipitation, carefully worked out and frequently repeated will allow the isolation of pure fractions but such methods are not at present satisfactory as analytical procedures.

Tiselius (3) has developed an apparatus for the study of the migration of protein in the electric field, eliminating most of the difficulties previously encountered in such studies and permitting a quantitative estimation of proteins as they separate during electrophoresis by means of a Toepler Schlieren method (4). With this apparatus he has studied among other things the proteins of horse serum (5, 6). The simplicity of the method, the sharp separation of the fractions, and the small amounts of material required combine to make it an excellent procedure for the study of human proteins.

Stenhagen (7) first pointed out that the principal electrophoretic fractions of normal human serum corresponded with those noted by Tiselius for horse serum namely albumin and α , β , and γ globulins migrating with varying speeds in the electric field. Later Blix (8) studied normal human serum globulins and those of patients with pneumonia, and showed that the so-called α and β fractions were usually increased during the acute phase of the disease. Recently, MacInnes and Longworth (9) and Longworth, Shedlovsky and MacInnes (10) have reported their observations on serum and urinary proteins in a number of diseases. They have noted that the urinary protein pattern in nephrosis resembles normal serum, in spite of great variations in the serum albumin and globulins. In a number of febrile conditions they observed elevation of α -globulin, and in myeloma, nephrosis and obstructive jaundice large increases of the β globulin fraction associated in the latter two diseases with an increase in blood lipids. In the present study of the proteins of normal and pathological plasmas and urines the globulins have been analyzed into four fractions by electrophoresis at neutral reactions and the albumins into two fractions at acid reactions.

METHODS

The principles involved in the Tiselius apparatus are simple. The plasma is layered in the lower half of a U tube below buffer solution (against which the plasma has previously been dialyzed). Electrodes are placed in the buffer and direct current applied. The voltage and current are adjusted so as not to produce appreciable warming of the solution and consequent convection currents. This disturbance is minimized by running the apparatus in a bath at 1° C. a temperature just below the point of maximum density of the solutions used. Under the influence of the electric field the protein ions move toward the anode if their charge is negative and toward the cathode if their charge is positive. The speed of migration is largely dependent on the charge of the protein ion under conditions of constant pH and concentration. The various fractions combined

TABLE II
Percentage composition of proteins of normal and certain pathological fluids

	Total protein	Albumin	Globulins					Fibrinogen	A/G Tiselius	A/G Howe
			α	β_1	β_2	β_{total}	γ			
	<i>in grams per 100 cc</i>	<i>per cent</i>	<i>per cent</i>					<i>per cent</i>		
NORMAL PLASMA										
Average	6.5	62.5	7.0	4.8	8.4	13.2	11.6	5.7	63/37	62/38
NEPHROTIC SYNDROME										
Plasma I	4.0	27.4	15.2	15.8	18.4	34.2	4.8*	18.4	27/73	43/57
Urine I	1.0	92.0	1.7			5.3	1.0		92/8	
Plasma III	4.2	17.2	31.7			33.0	5.6	12.5	17/83	26/74
Urine III	0.6	83.3	4.5			10.4	1.8		83/17	
Plasma IV	3.9	8.7	29.7			41.6	6.8	13.2	9/91	23/77
Urine IV	0.4	67.7	5.9			15.2	11.2		68/32	
TERMINAL NEPHRITIS										
Plasma V	6.0	51.8	5.5	6.7	15.3	22.0	14.2	6.5	52/48	62/38
Urine V	0.2	68.7	3.4			14.7	13.2		69/31	
Plasma VII	6.6	64.9	5.4	4.7	10.8	15.5	9.0	5.2	65/35	68/32
Urine VII	0.15	80.5	3.1			8.3	8.1		81/19	
AMYLOID DISEASE										
Serum VIII	5.0	26.2	16.7	10.2	18.7	28.9	28.2		26/74	42/58
Urine VIII	0.4	72.7	1.5			5.2	20.6		73/27	
ACUTE RHEUMATIC FEVER										
Plasma IX	5.4	31.1	10.4	10.0	6.7	16.7	33.5*	8.3	31/69	39/61
Urine IX	0.05	40.0	6.0			24.0	30.0		40/60	
CIRRHOSIS										
Plasma XII	5.7	39.3	5.8	4.8	9.4	14.2	32.0	8.7	39/61	44/56
Ascites XII	0.6	41.1	4.5			13.8	34.5	6.1	41/59	

* The γ -fractions in these two cases include P_2 . In Case I, P_2 represented 2.1 per cent. In Case IX, it represented 7.4 per cent.

2 Nephrotic syndrome The patients studied were three children and one adult presenting

	Svensson	Longworth	Luetscher	Kekwick
α /Albumin	0.13	0.12	0.11	0.08
β /Albumin	0.26	0.23	0.21	0.19
γ /Albumin	0.17	0.20	0.19	0.43

The high γ value observed by Kekwick (30) is due to the overlapping δ -effect. The results of Blux are difficult to compare directly but appear to resemble those of Kekwick. A good agreement of these results is quite gratifying.

gross proteinuria, hypoproteinemia, and edema, without significant change of renal function or blood pressure.

The most striking change is the great loss of albumin from the plasma, which is even more severe than indicated by the ratio of albumin to globulin (A/G ratio), as measured by Howe's method. Compensatory changes involve the increases of β - and α -globulins, β increasing first, and α rising strikingly only when the albumin is

almost totally lost.³ The γ -fraction least active osmotically is diminished. Fibrinogen is definitely increased, accounting at least in part for the high corpuscular sedimentation rates observed in nephrotic blood. In one case P_2 appeared and was apparently related to increase of true globulin and diminution of γ .

The urines contain roughly the same fractions as normal serum. Fibrinogen is not ordinarily seen, nor is P_2 . The β boundary is single. This is perhaps explained by the fact that fibrinogen and the more insoluble globulins are precipitated from dilute solutions of pH 5.5 to 6.5. It is known that urinary 'casts' are soluble in alkaline solutions.

When there is over 1 gram per cent of albumin in the plasma, the urinary protein is about 90 per cent albumin. When the albumin in the plasma falls to very low levels the urinary albumin becomes lower and the urinary globulin relatively more important, approaching a ratio similar to that of normal serum as has been pointed out by MacInnes and Longworth (9).

3 Terminal glomerulonephritis The three cases studied were all in a state of uremia. One gave a history of acute glomerulonephritis with persistent activity; one had an insidious onset with an interlude of mild edema; one had gone through an acute attack, a nephrotic stage and a latent period of 10 years.

Analyses of the three plasmas approximated the normal. There was a slight diminution of the albumin in two cases and the β -globulin was somewhat higher than usual in all cases.

Urines contained lower concentrations of protein than the nephrotics but much the same proportion of constituents. Here again diminution of plasma albumin concentration is reflected in a lower proportion of albumin in the urine.

4 Amyloid disease Only one patient was studied. Chronic tuberculosis of the lymphatic system preceded the development of amyloidosis in this case. During life the patient presented a classical picture of amyloid disease and the diagnosis was confirmed by necropsy findings.

The albumin was much reduced in plasma, but unlike a nephrotic serum with a similar diminu-

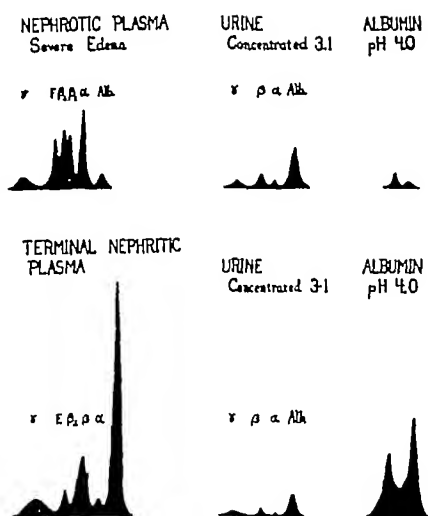


FIG. 2. SCHLIEREN DIAGRAMS REPRESENTING THE DISTRIBUTION OF PROTEIN FRACTIONS IN THE PLASMA AND URINE OF PATIENTS WITH GLOMERULONEPHRITIS IN THE NEPHROTIC AND TERMINAL STAGES.

A very severe reduction of albumin characterizes the nephrotic plasma shown above. Corresponding to this situation, the urine contains less albumin and more globulin than seen in the milder case. The ratio of albumin components is reversed in both blood and urine. The plasma of terminal glomerulonephritis is more nearly normal, but shows mild changes of the same type seen in the nephrotic stage.

tion of albumin the globulin increase was most striking in the γ -fraction. The urine reflected this anomaly with a corresponding increase in the γ fraction otherwise resembling the other urines studied.

5 Rheumatic heart disease with failure Several patients in the active phase of rheumatic fever and one patient without obvious signs of activity were studied. In a young girl with rheumatic pancarditis there was a great elevation of the γ -fraction. There was also a decrease of P_2 and an increase in β_2 (bresis app. lins). Fibrinogen was relatively and at patients studied. The albumin was considerably reduced in plasma. In a woman with rheumatic pericarditis types have been noted similar changes but serum albumin has shown two

A reversal of the normal

³ Longworth and MacInnes in a recent paper (31) relate this increase in β globulin to lipoids.

ratio of these components has been noted in the nephrotic syndrome and in advanced cirrhosis of the liver

3 The ratio of nephrotic albumin components being the same in plasma and urine suggests a change in the formation of albumin

4 Results obtained with the salting-out and the electrophoretic methods of protein fractionation have been compared

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THE EFFECT OF UNILATERAL SPONTANEOUS PNEUMOTHORAX ON THE CIRCULATION IN MAN

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The effect of artificial pneumothorax on cardiac output in animals has been studied by several investigators. Dogs have not been suitable as experimental animals because of the flimsy and often perforated mediastinum. In both the rabbit and the goat the mediastinum is intact but the goat is the more satisfactory because of its size, larger blood volume, and the apparent similarity of its mediastinum to that of man. Among the recent work on the subject is that of Hilton (1). He selected goats as experimental animals and made use of the Fick principle for estimation of cardiac output. He found that a small unilateral pneumothorax gave rise to a slight increase in cardiac output but that a unilateral pneumothorax of moderate size resulted in a reduction in cardiac output of about 30 per cent. He suggested on the one hand that the initial increase in cardiac output was caused by the increased inspiratory efforts and probably anoxemia and on the other that the decrease after induction of larger pneumothorax was due to diminished mechanical efficiency of breathing and consequent interference with the venous return to the heart.

Several reports have been made on the effect that pneumothorax has on cardiac output in man. The subjects for most of these investigations have been patients suffering from parenchymal pulmonary disease usually pulmonary tuberculosis. Richards, Riley and Hiscock (2) studied three young men exhibiting unilateral pulmonary tuberculosis before and after the induction of unilateral pneumothorax. In measuring cardiac output they used a method employing the Fick principle. In all three patients they observed fall in level of cardiac output on institution of unilateral pneumothorax.

Cournand, Bryan, and Richards (3) used a method similar to the one employed by Richards, Riley and Hiscock (2) but exercised particular care in securing the venous and arterial carbon

dioxide values. They made measurements of cardiac output when the lungs were expanded as well as during various stages of collapse in three patients suffering from pulmonary tuberculosis, in one having a lung abscess and in two exhibiting spontaneous pneumothorax. In three instances unilateral pneumothorax was associated with substantial decrease in cardiac output. In the remaining three cases, however, there was no consistent relation between the cardiac output and the degree of collapse. Since however the two cases of spontaneous pneumothorax were not studied after the lungs had completely re-expanded control levels were not obtained in them. They inferred that in unilateral pneumothorax there was a tendency to diminished cardiac output which might be marked, slight or absent. They suggested that pulmonary blood flow might be related more to the degree of ventilation of the collapsed lung than to the degree of collapse and that the motion of the diaphragm might have an important influence.

Nylin (4) used the acetylene method of Grollman in studying two cases both before and at intervals during the collapse of one lung by induced unilateral pneumothorax. Both patients suffered from pulmonary tuberculosis. In neither case did he observe progressive or consistent change in the cardiac output as unilateral pneumothorax was established.

Prikryl (5) studied five patients in whom he used the method of Grollman for the estimation of cardiac output. The patients were afebrile when estimations were made and basal conditions were observed. In four cases studied before and after the collapse of one lung he found a slight decrease in cardiac output (8, 9, 6 and 1 per cent). In a fifth case, there was a slight increase (3 per cent). He considered these variations to be within the limits of normal fluctuation and ex-

perimental error and concluded that cardiac output was not decreased by collapse of one lung

The observations now being reported concern four patients who suffered unilateral spontaneous pneumothorax. Studies were made on them during various stages of collapse and after complete re-expansion of the lung. These patients appeared particularly suitable for this investigation because they were in good health when spontaneous pneumothorax occurred, because they had no evidence of pulmonary disease or organic heart disease, because there was no fluid in the pleural cavity, and because they were able to cooperate fully in carrying out the procedures. There were no signs of lung fistula. Studies were carried out during various stages of collapse of the lung and after complete re-expansion.

METHODS

All observations were made in the morning while the patients were in a basal metabolic state. Measurements of the cardiac output were made by the acetylene method, three samples of gas being taken as first recommended by Grollman (6), and as further elaborated by Grollman, Friedman, Clark and Harrison (7). During this measurement the patients were sitting in a steamer chair (angle 135°) with legs extended. They were acquainted with and trained to carry out the procedures beforehand. While the patient was at rest the cardiac rate was counted at intervals of five minutes. At the end of one hour the acetylene-air-oxygen mixture was rebreathed. Three samples of gas were taken during each rebreathing period for estimation of the arteriovenous oxygen difference. The first sample was taken after rebreathing ten to twelve times in twenty seconds, the second after two to three breaths more, and the third after two to three additional breaths. All three samples were usually obtained before the end of thirty seconds. Samples were taken during expiration. Two periods of rebreathing were carried out on each patient. Shortly afterwards the oxygen consumption was measured with a Benedict-Roth spirometer. After a short pause, the vital capacity was measured and height and weight determined. In succession, sufficient time being allowed between each procedure for the patient to return to a basal metabolic state, an electrocardiogram including a chest lead was taken, the arm-to-tongue circulation time recorded, the venous pressure estimated, and the blood pressure measured. Finally, the basal state still being maintained, a roentgenogram of the heart was made at a distance of two meters. The percentage of collapse of the lung was estimated by measuring with a planimeter the area of the shadow of the collapsed lung on the roentgenogram and the area of that part of the chest cavity normally filled by the lung when expanded. We realized that this method of esti-

imating percentage collapse of the lung was not exact since it did not take into account the changes in the other planes. It was considered satisfactory, however, for a rough correlation. The three standard leads of the electrocardiogram were taken in each instance, and also in the case of H H and H G electrocardiograms were recorded with the patient supine, lying on the right side and lying on the left side, in order to calculate the shift of the electrical axis with change in position.

The arm-to-tongue circulation time was estimated by the use of decholin (8). Five cc. of a 20 per cent solution were injected rapidly (one to two seconds) through an 18 gauge needle into an antecubital vein while the patient was lying quietly in the supine position. This was repeated one and one-half minutes after the response to the first test had been elicited. The time was recorded from the beginning of the injection until the patient perceived the bitter taste.

The venous pressure was measured by the direct method (9), using a large antecubital vein, the arm being placed on a level with the right auricle. The system was filled with normal saline and the venous pressure read directly from a millimeter rule as millimeters of saline. Normal pressures by this method range from 40 to 100 mm. of saline. In subsequent measurements the vein was entered at the site first punctured, the vein of one arm being reserved for measurement of venous pressure, and of the other arm for estimation of the circulation time.

Roentgenograms of the heart were taken with the patient in the standing position, in full inspiration, at a distance of two meters. Measurements of the cardiac area were carried out by the technique of Levy (10).

In one patient (T C) estimations of the oxygen content of the blood were made. Samples of arterial blood were taken under albolene from a radial or brachial artery, and of venous blood, without stasis, from an antecubital vein, the same vein being used for this purpose each time. The oxygen content of these samples was estimated by the Van Slyke and Neill manometric method (11). Samples of blood were taken in the morning before breakfast with the patient in a basal metabolic state.

OBSERVATIONS

The data are recorded in Table I.

Case H H was studied first when he showed 77 per cent collapse of the right lung, again when there was 34 per cent collapse, and finally after complete expansion (Table I). The cardiac output increased from 191 to 274 and finally to 30 liters per minute as the pneumothorax disappeared. The arteriovenous oxygen difference decreased. The arm-to-tongue circulation time decreased slightly. The venous pressure decreased slightly. Roentgenogram showed presence of the pneumothorax and slight displacement of the heart to the left. This displacement disappeared as the

TABLE I

The effect of unilateral pneumothorax on certain measurements of the circulation

History number	No. 21393			No. 70432			No. 21713			No. 163478		
Name, age, sex	F.H. 47 years ♂			T.C. 23 years ♂			H.H. 43 years ♂			H.Q. 29 years ♂		
Collapsed lung	Right			Right			Right			Left		
Date	No- vember 5, 1935	No- vember 14, 1935	De- cember 1935	No- vember 22, 1934	Janu- ary 12, 1934	Janu- ary 12, 1935	No- vember 5, 1938	No- vember 22, 1938	Feb- ruary 7, 1939	No- vember 25, 1937	April 13, 1939	
Body surface area, sq. m.	1.70	1.77	1.83	1.72	1.73	1.77	1.74	1.73	1.79	1.84	1.80	
Oxygen consumption, cc. per minute	210	205	195	209	216	211	207	197	201	223	234	
Basal metabolic rate, per cent	-2	-10	-17	-12	-9	-12	-9	-15	-16	0	-4	
Arteriovenous oxygen difference, cc.	77.1	57.1	60.6	63.4	75.9	64.5	107.9	73.0	88.1	75.3	87.3	
Cardiac output, liters per minute	2.80	3.50	3.13	3.20	3.81	3.25	1.91	2.74	3.00	3.63	3.43	
Cardiac output, liters per sq. m. per minute	1.57	2.03	1.77	1.86	2.21	1.85	1.10	1.54	1.70	1.93	1.93	
Heart rate, beats per minute	90	78	72	83	85	73	94	74	72	80	80	
Cardiac output per beat, cc.	31	46	44	39	45	45	20	37	43	34	44	
Cardiac area, sq. cm.	157.8	152.6	148.3	155.3	159.5	158.4	125.6	124.6	137.0	122.7	123.9	
Arterial pressure, mm. Hg	100/54	115/60	110/53	120/78	125/74	131/60	150/98	130/80	112/78	85/50	104/60	
Cardiac work per beat, gram. per beat	48.2	61.8	64.1	60.9	43.5	66.9	33.7	54.8	65.4	51.4	49.1	
Circulation time, seconds	20.4	19.3	19.0	12.6	12.4	17.3	12.8	11.8	9.8	7.8	12.1	
Venous pressure, cm. of saline	8.1	8.4	6.1	15.5	10.8	4.5	5.5	6.1	5.5	8.8	6.7	
Vital capacity, cc.	2000	2300	3000	2600	3000	3000	3000	3000	3500	2300	2500	
Right lung area, sq. cm.	220.5	255.3	300.7	182.4	174.5	227.8	68.3	207.8	264.7	246.5	255.5	
Pneumothorax area, sq. cm.	134.6	46.4	0	173.3	124.6	0	231.7	104.8	0	200.4	0	
Left lung area, sq. cm.	378.6	253.6	258.1	217.5	227.7	234.4	27	261.9	248.7	78.8	231.0	
Unilateral collapse, per cent	14	0	0	0	0	0	67	0	0	0	0	
Cardiac output, liters per minute per cent of control	90	113	100	98	68	100	64	91	100	87	100	
Cardiac output, per beat per cent of control	70	103	100	80	70	100	50	90	100	80	100	
Vital capacity, per cent of control	55	90	100	62	71	100	57	74	100	61	100	

lung expanded while the vital capacity increased from 2000 cc., to 2600 cc., to 3500 cc. Electrocardiograms taken with the patient supine, lying on the right side, and lying on the left side showed only slight changes in deviation of the electrical axis with change in position.

Case H G was studied first when the left lung was collapsed 73 per cent and again after complete expansion. The cardiac output increased from 3.03 to 3.48 liters per minute. There was a moderate decrease in the arteriovenous oxygen difference. The arm-to-tongue circulation time increased from 7.6 to 12.1 seconds. The venous pressure increased slightly. Roentgenogram showed no appreciable displacement of the heart. The vital capacity increased from 2300 cc. to 3800 cc. Electrocardiograms were taken with the patient supine lying on the right side and lying on the left side. There was a slight left axis deviation during the period of pneumothorax which later disappeared. However, the electrical axis shifted more with change in position during the period of pneumothorax than it did later.

Case F H was studied first with 38 per cent collapse of the right lung later with 14 per cent collapse and finally after complete expansion. The cardiac output increased from 2.80 liters per minute with 38 per cent collapse to 3.59 liters per minute with 14 per cent collapse and then

decreased to 3.12 liters per minute after the pneumothorax had disappeared. Unlike the other patients this case achieved his largest cardiac output while there was partial collapse of one lung. Arteriovenous oxygen difference also was lowest at the time of 14 per cent collapse of one lung. The arm-to-tongue circulation time did not vary appreciably. The venous pressure showed a moderate increase but remained within normal limits. The roentgenogram showed the pneumothorax on the right and a slight shift of the heart to the left that disappeared as the lung expanded. The vital capacity increased from 2000 cc. to 3200 cc. and finally to 3600 cc. Electrocardiograms consisting of the three standard leads and a chest lead showed a slight shift of the electrical axis to the right and a slight increase in the amplitude of the T-wave in the chest lead as the heart resumed its normal position.

Case T C was studied first when there was 57 per cent collapse of the right lung, again when there was 44 per cent collapse and finally after complete expansion. The cardiac output measured 3.20 liters per minute with 57 per cent collapse decreased to 2.81 liters per minute with 44 per cent collapse and increased to 3.28 liters per minute after complete expansion. Unlike the other patients, this case had a smaller cardiac output when there was a moderate degree of col-

lapse than when there was more extensive collapse. The arteriovenous oxygen difference increased and then decreased as the lung expanded, being greatest at the time of 44 per cent collapse. The arm-to-tongue circulation time decreased slightly and then increased as the pneumothorax decreased but stayed within normal limits. The venous pressure was definitely elevated at the time of 57 per cent collapse but showed a substantial fall at each of the subsequent examinations. The roentgenogram showed a slight shift of the heart to the left at the time of maximum collapse but it was seen to be in normal position as pneumothorax decreased. The vital capacity increased from 2600 cc, to 3000 cc, and finally to 4200 cc. Electrocardiograms consisting of the three standard leads and a chest lead showed a slight shift of the electrical axis to the left as the heart returned to its normal position with expansion of the lung.

DISCUSSION

The decrease in cardiac output for individual patients showed no close correlation with the amount of pneumothorax. Two patients showed a moderate decrease in the presence of unilateral collapse, another achieved a larger cardiac output with a small pneumothorax than was present either in the control period or with greater collapse, yet another patient attained a smaller cardiac output with a moderate collapse than when there was more extensive pneumothorax. In all patients the basal metabolic rate was normal but on the low side of normal. In two patients (T C and H G) it changed only slightly as percentage collapse decreased. Case F H, however, exhibited a fall from —2 per cent to —10 per cent to —17 per cent as the lung expanded, and Case H H showed a decrease in the basal metabolic rate from —9 per cent to —15 per cent. This change in the basal metabolic rate paralleled the slowing of the heart rate which occurred in these two cases. The fall in basal metabolic rate in these last two cases (F H and H H) accounted to some extent for the absence of a more substantial increase in their cardiac output. Nevertheless when percentage collapse was plotted against per cent of control cardiac output, for all patients, a rough linear correlation was seen, and the slope of the line was such that the greatest

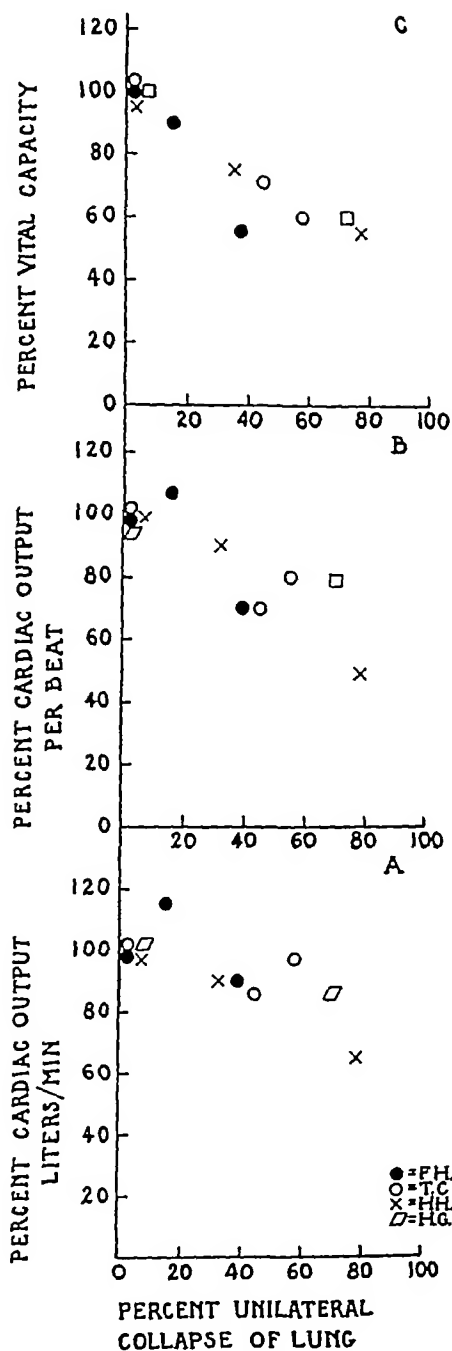


FIG. 1 COLLAPSE OF LUNG AND CARDIAC OUTPUT AND VITAL CAPACITY

In this figure all observations of cardiac output per minute (A), output per beat (B), and vital capacity (C) as per cent of the levels when the lung had expanded completely, are plotted against the corresponding per cent collapse of the lung (Table I). This discloses a correlation which appears to be linear in that, as the amount of collapse increases, the cardiac output and vital capacity decrease.

collapse was associated with the lowest cardiac output (Figure 1). The small collapse which was associated with increase in cardiac output will be discussed later. Similarly there was a rough correlation between cardiac output per beat and amount of collapse, since the cardiac output per beat increased as the per cent of collapse decreased. There was no constant correlation between arteriovenous oxygen difference and degree of pneumothorax. The heart rate in each of the patients became slower as the pneumothorax disappeared. The arm-to-tongue circulation time showed no consistent change: in one case there was no change in two there was an increase, and in the fourth there was a slight decrease as the lung expanded. The venous pressure was within or near normal limits and showed only slight changes except in the case of T C in whom there was a considerable fall from 16.3 cm to 4.8 cm of saline. The vital capacity varied inversely with the degree of collapse, and the correlation appeared to be linear (Figure 1).

Since the cardiac output appears to be reduced when a sufficiently large unilateral collapse of a lung occurs by what mechanisms does the organism compensate for this reduction? There are data relating to one mechanism in the case of one patient. Measurements of the oxygen content of the arterial and venous blood were made of T C. During collapse the arterial oxygen saturation was reduced to 92.3 per cent and 93.5 per cent respectively (Table II), and restoration to a normal level namely 97.5 per cent, occurred with complete expansion of the lung. The reduction in saturation during collapse may be accounted for by the mixture of blood of reduced

oxygen content leaving the partially collapsed lung with normally oxygenated blood from the opposite normal lung. One of the mechanisms achieved by this patient was to raise the oxygen-carrying capacity of the blood by increasing the amount of hemoglobin as much as 8 to 12 per cent (Table II) over what it was after re-expansion of the lung. It was possible for him to maintain his normal range of oxygen utilization during collapse (Table II).

Although roentgenograms showed slight displacement of the heart to the side opposite the pneumothorax in each case the electrocardiograms showed no consistent deviation of the electrical axis. Moreover as the pneumothorax disappeared and the heart returned to normal position the electrical axis shifted in a direction similar to that of the heart in two cases but in the opposite direction in the other two cases. Rotation of the heart which could not be demonstrated on anteroposterior roentgenograms doubtless accounted for this inconsistency.

In only one of the patients (Case F H) were studies of the circulation performed in the presence of a unilateral pneumothorax as small as 14 per cent. The results here were similar to those found by Hilton (1) in his observations on goats in that there was an increase in cardiac output with this small amount of collapse of one lung. The patient showed neither cyanosis nor dyspnea but the rate of respiration was slightly more rapid at the time of this measurement than after the pneumothorax had disappeared. From our data and those reported in the literature, it appears that in the presence of a unilateral pneumothorax of small or moderate size, there is likely

TABLE II
Changes in blood gases during unilateral collapse in T C

Date	Unilateral collapse	Oxygen content arterial blood	Oxygen content venous blood (arm)	Oxygen capacity venous blood	Hemoglobin†	Arterial oxygen saturation	Venous oxygen saturation	Arterio-venous oxygen difference	Oxygen content mixed venous blood*	Oxygen utilization‡
	per cent	cc. per liter	cc. per liter	cc. per liter	per cent	per cent	per cent	cc. per liter	cc. per liter	per cent
November 23 1934	57	209.0	163.0	229.0	112.4	92.3	71.2	63.4	145.6	28
November 30 1934	44	218.0	127.0	236.0	116.2	93.5	52.1	76.9	131.1	33
January 12 1935	0	206.0	138.0	215.0	104.9	97.5	63.4	64.5	141.5	30

* Derived by subtracting arteriovenous oxygen difference from oxygen content of arterial blood

† Ratio between arteriovenous oxygen difference and oxygen capacity

‡ 185 cc. per liter = 100 per cent hemoglobin

TABLE I
Composition of diets in grams per day
 (Values for calcium, phosphorus, and nitrogen are actually analyzed)

	Case 1a		Case 2a	Case 3a'			Case 4a		
Periods (four-day)	1-5	6-11	1-12	1-2 10-14 16-17	3-9	19-20	1-10	11-16	17-26
Diet number	1	2	1*	1	2	3	3	4	5
Rice	50	62	180	150	100	460	190	190	190
Wheat flour	150	188	300	500†	400		150	300	400
Corn meal			100						
Millet	50	62					80	80	80
Oat meal	30	38							
Egg	30	38				90			
Beef	50	62	30						
Pork	100	125	30	30	30	50	100	100	100
Chicken	50	62		30	30	60			
Soy bean curd					100	200			
Gram bean					50				
Mung bean						20			
Potato, sweet							100		100
Lotus root starch				10	10				
Turnip					100		100	100	100
Turnip, salted			40			10			
Cucumber, salted							10	10	10
Wosun				100					
Cabbage, large			100	50			300	400	
Cabbage, small	50	62				100			400
Celery					100				
Spinach						100			
Bean sprouts	100	125		100					
Hai tai					20				
Apple						50			
Banana					100				
Crabapple					50				
Pear	100	125		100					
Tangerine							100	100	100
Molasses					40				
Sugar	10	12	20	20	20	20			
Butter	10	12							
Sesame oil	42	52	30	50	50	60	50	50	50
Sauce, soybean				10	10	10			
Sodium chloride	6	7.5	5	6	9	8	10	10	12
Baking powder				20‡	20‡				
Protein	80	100	74	86	88	102	70	88	100
Carbohydrate	234	293	479	557	509	411	374	489	561
Fat	82	103	35	62	65	88	73	75	76
Calories	1994	2499	2528	3125	2972	2844	2435	2980	3329
Calcium, mgm	247	309	227	186	505	421	207	292	456
Phosphorus, mgm	1025	1281	932	930	1157	1174	812	1065	1006
Nitrogen	11.58	14.50	11.34	12.35	13.76	16.25	10.61	12.96	14.84

* Half portion of this diet was used for periods 2-4, and two-thirds portion for periods 5-6 and 10-12 inclusive

† Only 400 grams of flour were used for periods 10-14, and 300 grams for periods 16-17, inclusive. Flour on analysis gave 21 mgm calcium, 133 mgm phosphorus, and 1.63 grams nitrogen per 100 grams

‡ Baking powder on analysis contained 986 mgm calcium, and 1,587 mgm phosphorus in 20 grams. This was used during periods 7-9 (diet 2) and 10-11 (diet 1) only, accounting for the exceedingly high intake of calcium and phosphorus during those periods

with tetany and rickets, breast-fed by its mother who also had tetany as well as osteomalacia, and the changes brought about both in the infant and in the mother after the latter alone received vitamin D

3 The response of an infant, born of an osteomalacic mother and itself having tetany and rickets, to breast feeding from a presumably normal

wet nurse before and after vitamin D administration to the wet nurse

4 The effect on an infant of variations in the state of vitamin D nutrition in the mother who had osteomalacia: a limited dose of vitamin D during the latter part of pregnancy, depletion for a period after parturition, and finally replenishment

PROCEDURE

All the patients were studied in the metabolism ward where the routines for the preparation and serving of constant diets and collection of excreta for quantitative purposes have been standardized (7 13 14). The composition of the diets is shown in Table I. While the fuel values are calculated from the compilation of Wu (25) and from *Outline of Diets* of the Peking Union Medical College Hospital 3rd edition, Peking 1937 the calcium, phosphorus and nitrogen contents were analyzed, 50 per cent of the day's food being used for the purpose. Where the same diet was used for a relatively long period of time, the analyses were repeated at intervals.

Stools and urine were collected in four-day periods 0.1 gram of carmine being used to mark off the stools every four days. For the quantitative collection of excreta from infants the special metabolism cots, described by Tso (23) were used. As all the infants studied were male, the urine collection was satisfactory and complete. It is usually a good plan, if time permits, to interpose periods of rest between periods of study so as to avoid maceration of the skin of the perineum and possible ill effects of continuous restriction of activities of the infant.

Stools were passed directly into tared enamel pans, dried in an oven at 90 to 100 C., and weighed. All the

stools of the period were ground in a mill into a fine homogeneous powder and accurately weighed portions ashed in a muffle furnace at approximately 500 C. The ash was extracted with hot 5 per cent hydrochloric acid and portions taken for analysis of calcium and phosphorus. The four-day pooled collection of urine was preserved by the addition of 5 cc. of concentrated hydrochloric acid per liter and analyzed for the same elements.

Breasts were emptied five or six times every twenty four hours by means of a hand or electric breast pump. A measured amount of each milking was fed to the infant from a feeding bottle and a small aliquot was saved. The pooled specimens of one or four days were used for analysis. Venipunctures were done before breakfast at the beginning of each period, but less frequently in the case of infants.

The analytical procedures for calcium and phosphorus in stool, urine, blood, milk and food have been given previously (7 13 14). The macro-Kjeldahl method was used for nitrogen determinations.

RESULTS

Composition of milk All the lactating women included in this study were good milk providers

TABLE II
Composition of breast milk

Four day period	Case 1a				Case 2a				Case 3a				Case 4a			
	Vol	Calc.	Phos-	Ni	Vol	Calc.	Phos-	Ni	Vol	Calc.	Phos-	Ni	Vol	Calc.	Phos-	Ni
	ume	ium	phorus	trogen	ume	ium	phorus	trogen	ume	ium	phorus	trogen	ume	ium	phorus	trogen
	cc.	mgm. per cent	mgm. per cent	mgm. per cent	cc.	mgm. per cent	mgm. per cent	mgm. per cent	cc.	mgm. per cent	mgm. per cent	mgm. per cent	cc.	mgm. per cent	mgm. per cent	mgm. per cent
1	819	30.95	22.97	277.9	922	35.30	16.21	180.5	558	32.28	13.69	217.4	498	25.74	16.51	241.0
2	936	30.67	22.83	274.8	952	31.48	15.00	154.7	623	34.20	14.32	215.0	581	27.90	16.85	232.6
3	1041	29.68	20.62	259.9	904	33.20	14.88	167.0	654	34.18	14.25	221.7	680	27.80	17.85	232.6
4	1116	30.56	19.52	249.5	877	34.20	15.36	203.0	739	30.76	15.62	221.4	780	26.80	17.02	226.7
5	1146	30.62	18.64	225.4	907	32.34	15.68	167.0	798	30.96	13.95	206.6	867	30.16	16.44	215.2
6	1230	31.30	17.45	226.0	908	31.80	16.83	177.5	837	33.39	13.28	213.8	928	30.40	17.12	212.3
7	1236	32.06	17.76	222.8	906	29.00	15.84	165.2	870	30.78	12.38	203.0	981	29.30	16.00	205.3
8	1274	31.35	17.16	222.7	902	31.98	14.98	188.6	794	28.04	12.48	199.7	1009	27.80	16.00	198.0
9	1309	31.70	16.25	214.0	906	32.07	16.20	197.5	800	28.63	11.68	199.0	1070	29.50	14.36	192.0
10	1353	31.46	16.38	210.3	976	32.52	15.70	189.8	927	33.68	11.18	194.0	1079	29.25	14.52	185.2
11	1367	30.32	16.00	210.2	1030	31.94	14.90	190.0	1016	30.83	13.06	194.0	1145	27.89	14.02	170.8
12					1118	32.20	15.51	165.3	1007	33.59	12.08	196.9	1184	27.30	13.96	172.2
13									980	29.85	11.88	181.5	1175	29.60	14.68	176.4
14									946	31.15	11.50	176.0	1183	29.40	13.45	168.5
15									966	30.34	11.63	183.3	1200	28.96	14.70	165.6
16									992	28.60	11.50	184.3	1229	27.63	13.08	161.4
17									996	29.81	12.60	176.0	1256	27.19	13.15	156.8
18													1248	25.02	12.84	148.0
19									1087	27.90	13.42		1248	26.09	12.14	157.8
20									1132	27.09	12.28		1248	25.63	12.28	160.0
21									1066	27.00	11.44	152.0	1268	24.41	12.34	164.8
22									1008	27.69	11.48	159.0	1156	26.06	12.31	161.6
23													1236	26.59	12.91	169.9
24													1138	25.29	12.56	159.1
25													1251	25.09	11.92	157.3
26													1310	22.53	12.46	145.2

Note. Studies of milk started eighteen days postpartum in Case 1a, two months in Case 2a, one month in Case 3a and seventeen days in Case 4a. Values are expressed as daily averages for each four-day period.

They were all in early stages of lactation. The milk yield, starting from 500 to 900 cc per day, gradually increased so that eventually it exceeded 1 liter in Cases 2a and 3a' and 1.3 liters in Cases 1a and 4a (Table II). The average calcium content varied from 27 to 32 mgm per 100 cc in the 4 cases. Slight fluctuations in calcium content from period to period were noticeable, but these could not be definitely correlated with progression of time, with the institution of vitamin D, or with the different levels of calcium intake. However, a descending tendency with the lapse of time was discernible in both the phosphorus and nitrogen content, particularly in Cases 3a' and 4a, in which the studies extended over eighty-eight and one hundred and four days respectively. The average phosphorus content was from 13 to 18 mgm per 100 cc and the average protein was from 1.7 to 2.2 per cent. The figures for calcium and phosphorus were within the limits of normal variation in the composition of human milk as compiled by Letch (11).

Case 1a Mother Y W L While anatomical evidence of osteomalacia was still present during this study, the disease was considered to be at the healing stage due to the large intake of calcium, phosphorus and vitamin D given during the last seven months of pregnancy. Studies began eighteen days postpartum. As seen from Figure 1 and Table III, calcium intake had to be raised to over 2 grams before a slight positive balance could be obtained. As discussed in a previous communication (12), this rather "extravagant" behavior was not the result of vitamin D deficiency, as Vigantol given during periods 7 to 11 made no difference to the calcium balance. It was considered very likely that the physiological requirements during active lactation rendered conservation very difficult and that the large amounts of calcium gained during pregnancy made further storage less urgent than in cases with less adequate preparation during pregnancy. Phosphorus metabolism was parallel to calcium metabolism. Serum calcium and inorganic phosphorus remained within low normal levels throughout the period of observations.

Case 1b Infant Y W L This infant was normal in every way. He was fed solely on the mother's milk. Metabolic observations were made for 7 four-day periods (periods 3 to 9). The intake of calcium increased progressively from 280 to 363 mgm per day as milk consumption increased (Figure 1 and Table IV). The urinary and fecal excretion of calcium was equal in extent, and both were minimal so that marked positive calcium balances were obtained, the average daily retention amounting to 74 per cent of the intake with very little variation from period to period. The same may

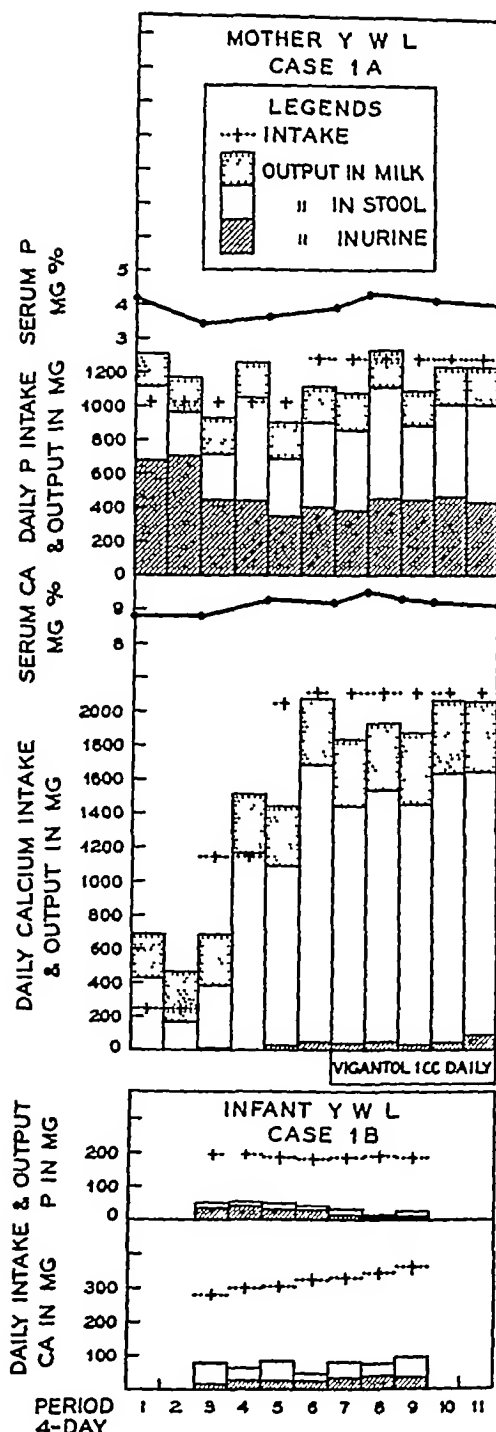


FIG 1 CALCIUM AND PHOSPHORUS METABOLISM OF CASES 1A AND 1B

The lactating mother had healing osteomalacia with adequate prior store and subsequent administration of vitamin D. The infant exhibited remarkably good retention of calcium and phosphorus throughout the periods of observation.

TABLE III

Mother Y W L Case 1a Calcium, phosphorus and nitrogen metabolism in a case of healing osteomalacia during lactation with adequate vitamin D supply

Date 1935	Period four day	Calcium, average per day					Phosphorus, average per day					Nitrogen, average per day					Serum	
		In-take	Urine	Stool	Milk	Balance	In-take	Urine	Stool	Milk	Balance	In-take	Urine	Stool	Milk	Balance	Calcium	Phosphorus
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm per cent	mgm per cent
IV30-V3	1	247	1	427	253	-434	1025	681	440	188	-284	11.58	7.01	1.26	2.28	+1.03	8.76	4.23
V 4-7	2	247	1	178	287	-219	1025	702	265	213	-155	11.58	7.06	0.93	2.57	+1.02		
V 8-11	3	1147	11	370	308	+458	1025	422	274	214	+115	11.58	7.33	0.64	2.70	+0.91	8.80	3.40
V12-15	4	1147	0	1162	339	-354	1025	439	602	217	-233	11.58	7.65	1.14	2.78	+0.01		
V16-19	5	2047	25	1062	351	+609	1025	348	341	214	+122	11.58	6.64	1.08	2.59	+1.27	9.23	3.60
V20-23	6	2109	46	1635	385	+43	1281	398	505	215	+163	14.50	7.89	1.60	2.78	+2.23		
V24-27	7*	2109	40	1398	396	+275	1281	382	473	219	+207	14.50	8.82	1.43	2.78	+1.47	9.20	3.88
V28-31	8	2109	51	1483	399	+176	1281	450	663	218	-50	14.50	9.72	1.72	2.84	+0.22		
VI 1-4	9	2109	30	1422	416	+241	1281	444	435	212	+190	14.50	9.43	1.45	2.80	+0.82	9.32	
VI 5-8	10	2109	44	1591	426	+48	1281	461	548	222	+50	14.50	9.26	1.71	2.87	+0.66		
VI 9-12	11	2109	85	1544	417	+66	1281	434	576	219	+52	14.50	9.40	1.78	2.90	+0.42	9.07	3.99

This table is reproduced from Chinese J. Physiol. 1937 11 292 (12) for convenience

* Vigantol 0.6 cc. daily was started from this period.

also be said of the phosphorus metabolism, the average daily retention being 80 per cent. Nitrogen balance was likewise positive, approximately 50 per cent of the intake being retained. Both the calcium and phosphorus balances were much more than normal, as discussed later.

The results on this infant show the remarkable manner in which the mineral elements of human milk were utilized with very little wastage. It is very likely that the vitamin D present in the milk, together with the store of the vitamin acquired during the antenatal period, was responsible for the extreme degree of conservation in calcium and phosphorus exchange exhibited by the infant. Extra ingestion of Vigantol by the mother did not further improve the degree of mineral conservation.

Case 2a. Mother C S Y This patient had tetany and mild osteoporosis of the visualized long bones at the time of metabolic observation. As shown in Figure 2 and Table V while on a minimal calcium intake of 227

mgm. per day the patient exhibited a moderately negative balance (period 1). With the intake raised to over 900 mgm. the loss of calcium was rectified (periods 2 and 3) but no substantial retention of the element took place until vitamin D was given (periods 4 to 12). The first 2 periods of Vigantol administration (1 cc. per day equivalent to 12,000 international units) were without effect, but from the third period on, retention amounted to from 30 to 40 per cent of the intake. This retention was brought about entirely by a reduction in the fecal calcium output, the secretion of calcium in the milk being maintained. At the height of vitamin D action (periods 10 to 12) small amounts of calcium appeared in the urine, indicating that intestinal absorption had improved to such an extent as to be more than sufficient for the needs of the body.

Phosphorus balance was markedly negative in the beginning but with vitamin D administration considerable

TABLE IV

Infant Y W L Case 1b Calcium, phosphorus and nitrogen metabolism of a presumably normal infant fed exclusively on mother's milk

Date 1935	Period four day	Breast milk intake	Calcium, average per day					Phosphorus, average per day					Nitrogen, average per day					Body weight
			In-take	Urine	Stool	Balance	Retention	In-take	Urine	Stool	Balance	Retention	In-take	Urine	Stool	Balance	Retention	
			cc.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	
V 8-11	3	944	280	18	60	+202	72	195	35	15	+145	74	2.45	1.03	0.24	+1.23	51	3.82
V12-15	4	985	301	28	36	+237	79	192	42	11	+139	72	2.46	1.18	0.12	+1.16	47	3.97
V16-19	5	995	305	26	60	+219	72	185	30	18	+137	74	2.24	0.93	0.27	+1.04	46	4.13
V20-23	6	1034	324	26	20	+278	86	180	23	7	+150	83	2.34	1.25	0.10	+0.99	42	4.30
V24-27	7*	1027	329	36	47	+245	75	182	15	17	+150	82	2.28	1.02	0.19	+1.07	47	4.45
V28-31	8	1102	346	41	36	+269	78	189	6	9	+174	92	2.44	1.09	0.11	+1.24	51	4.73
V11-4	9	1142	363	36	62	+265	73	185	10	17	+158	85	2.45	1.12	0.11	+1.22	50	4.97

* Mother started to receive renewed vitamin D supply

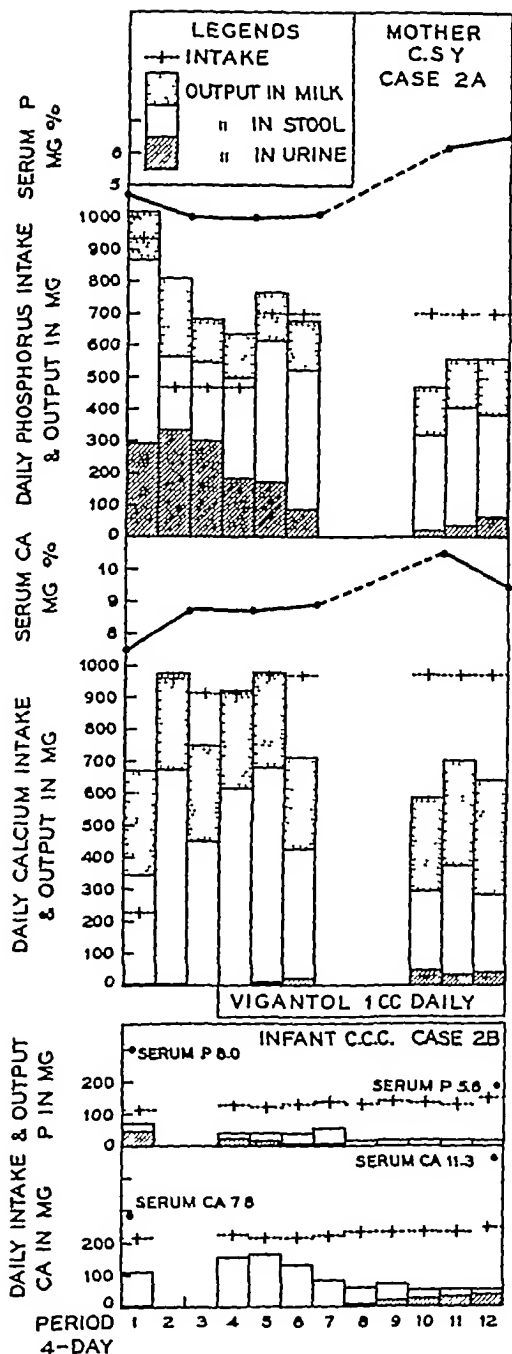


FIG. 2. CALCIUM AND PHOSPHORUS METABOLISM OF CASES 2A AND 2B

The lactating osteomalacia mother showed poor calcium and phosphorus balances which were subsequently improved by Vigantol therapy. A similar state of affairs was reflected in the infant (with rickets and tetany) via breast milk.

amounts of phosphorus were stored. The conservation of phosphorus, in contrast to that of calcium, was brought about mainly by a diminution in the urinary phosphorus output. Serum calcium, rather low to start with, was raised to normal after vitamin D therapy, and inorganic phosphorus was increased from a normal level on admission to a level higher than normal toward the end of the experiment.

This patient, then, differs from the first patient in that vitamin D therapy was capable of conserving the calcium and phosphorus metabolism so that not only were the extensive requirements of active lactation covered, but also considerable amounts of these minerals were retained in her own tissues and skeleton, which were probably in urgent need of reparation.

Case 2b Infant C C C The metabolism of the infant (Figure 2 and Table VI) reflects that of the mother. He was likewise admitted for tetany with evidence of rickets. The calcium retention of 48 per cent (period 1), as compared with that of the first infant, was poor, and it remained so during periods 4 to 6 when the mother began to receive vitamin D. But from period 7 on, the fecal output of calcium showed progressive reduction so that retention attained 80 per cent, comparable to the best performance of the first infant. The reduction of fecal calcium was accompanied by the appearance and increase of calcium in the urine.

Phosphorus balance was small in the beginning but it was very much increased after vitamin D therapy. The average retention of phosphorus during the last 5 periods was 88 per cent of the intake. Nitrogen retention, however, remained around 50 per cent. Serum calcium increased from 7.8 mgm at the beginning to 11.32 mgm at the end of the experiment, while inorganic phosphorus decreased from 8.0 mgm. to 5.61 mgm. The rachitic bone changes present on admission, however, were not altered on discharge, the period during which the infant was fed the breast milk of the mother receiving vitamin D being only thirty-six days.

Although on roentgenologic examination there was no obvious evidence of healing in the rickets of the infant within thirty-six days, the metabolic defects characteristic of rickets and tetany were corrected. This shows that the amount of vitamin D given to the mother was sufficient to enrich her milk and to render it effective in correcting the faulty mineral metabolism of the infant. Were the period of observation extended, healing of the rachitic bone changes would have followed eventually.

Case 3a' Wet nurse W C S This was presumably a normal lactating woman. Her metabolic behavior (Figure 3 and Table VII) may be taken to represent a normal state of affairs during lactation and it is closely comparable to that of Case 1a with healed osteomalacia. Marked negative balances in calcium were obtained on intakes of from 186 to 505 mgm per day (periods 1 to 6). It was only after the calcium intake was raised to about 1,500 to 2,000 mgm. that slightly positive balances began to appear (periods 7 to 11). Vitamin D in the form of Vigantol 1 cc. or 12,000 international units per

TABLE V

Mother C S Y Case 2a Calcium phosphorus and nitrogen metabolism in a case of tetany and osteomalacia during lactation before and after vitamin D therapy

Date 1937	Period four-day	Calcium average per day					Phosphorus, average per day					Nitrogen, average per day					Serum	
		In take	Urine	Stool	Milk	Balance	In take	Urine	Stool	Milk	Balance	In take	Urine	Stool	Milk	Balance	Calcium	Phosphorus
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm per cent	mgm per cent
IV 6-9	1	227	6	340	326	-445	932	296	571	150	- 85	11.34	5.92	2.31	1.67	+1.44	7.46	4.70
IV10-13	2	958	5	671	300	- 18	466	336	331	143	-344	5.67	5.99	1.42	1.47	-3.21		
IV14-17	3	914	4	446	300	+164	466	302	245	134	-215	5.67	4.24	0.57	1.51	-0.65	8.72	4.05
IV18-21	4*	914	4	613	300	- 3	466	183	317	135	-169	5.67	4.66	0.57	1.89	-1.45		
IV22-25	5	970	9	674	293	- 6	699	172	442	142	- 57	8.52	5.44	0.96	1.51	+0.61	8.70	4.03
IV26-29	6	970	21	407	288	+254	699	89	436	153	+ 21	8.52	5.27	1.85	1.61	-0.21	8.95	4.11
IV30																		
V12-15	10	970	46	223	318	+383	699	20	299	153	+227	8.52	5.09	0.97	1.85	+0.61	10.45	6.24
V16-19	11	970	34	342	329	+265	699	37	370	154	+138	8.52	5.22	1.44	1.96	-0.10	9.42	6.52
V20-23	12	970	38	244	360	+328	699	62	324	174	+139	8.52	4.70	1.50	1.85	+0.47		

* Vigantol 1 cc. started from this period

TABLE VI

Infant C C C Case 2b Calcium phosphorus and nitrogen metabolism of a breast fed rachitic infant and its response to vitamin D administration to the mother

Date 1937	Period four-day	Breast milk intake cc.	Calcium average per day					Phosphorus, average per day					Nitrogen average per day					Serum		Body weight kgm
			In take	Urine	Stool	Balance	Retention	In take	Urine	Stool	Balance	Retention	In take	Urine	Stool	Balance	Retention	Calcium	Phosphorus	
			mgm.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	mgm per cent	mgm per cent	
IV 6-9	1	656	232	5	105	+122	48	106	46	26	+ 34	31	1.18	0.61	0.24	+0.33	28	7.8	8.0	4.97
IV10-13	4*	648	247	0	156	+ 91	29	118	22	19	+ 77	65	1.34	0.33	0.26	+0.75	56			4.92
IV22-25	5	720	233	0	165	+ 68	29	113	16	25	+ 72	61	1.20	0.37	0.29	+0.54	45			4.97
IV26-29	6	720	229	0	131	+ 98	43	121	5	33	+ 83	68	1.28	0.43	0.29	+0.56	44			3.18
IV30-V3	7	810	235	5	79	+153	65	126	5	51	+ 72	56	1.34	0.38	0.26	+0.68	51			5.15
V 4-7	8	810	239	7	51	+201	78	122	2	13	+ 77	88	1.33	0.35	0.26	+0.92	60			5.34
V 8-11	9	810	260	18	51	+191	74	131	2	14	+115	88	1.60	0.44	0.32	+0.84	52			5.53
V12-15	10	810	264	25	28	+211	80	127	2	13	+112	88	1.54	0.50	0.28	+0.76	49			5.55
V16-19	11	810	259	31	27	+201	78	121	1	14	+106	88	1.54	0.45	0.27	+0.82	53			5.70
V20-23	12	900	289	32	24	+233	81	144	1	15	+128	89	1.48	0.43	0.30	+0.76	51	11.52	5.61	5.94

* Mother starting to receive Vigantol 1 cc. per day from this period on.

day was given from periods 5 to 15 and from periods 16 to 20 the dosage was raised to 1.5 cc. or 18,000 mter national units per day. All this vitamin D supply did not seem to produce obvious changes in the calcium metabolism, balances being approximately even on levels of intake varying from 1,000 to 2,000 mgm. No significant amounts of calcium appeared in the urine, the paths of excretion being mainly through the bowel and breasts during most of the periods. The absence of calcium in the urine was possibly related to the very high phosphorus intake (periods 7 to 11). While no substantial gain in calcium or phosphorus was evident after vitamin D administration, such therapy was probably important in enabling the subject to maintain mineral balance in spite of heightened requirements during lactation.

The phosphorus metabolism followed closely the calcium metabolism, although the urinary tract constituted by

far the largest channel of phosphorus elimination. Nitrogen gain prevailed during most of the experimental periods. Serum calcium and inorganic phosphorus showed no remarkable changes although a slight ascending tendency was discernible in both, as time went on.

From the observations on this experimental subject, it may be stated that a normal woman with intact skeletal calcification is able to maintain herself in calcium and phosphorus balance in the face of mineral stress incident to lactation provided that the level of intake is sufficiently high and vitamin D supply is adequate. However significantly positive balance is not expected as there is presumably no need for it.

Case 3b Infant T N T This infant, born of a mother having osteomalacia and tetany was admitted at the age of three months. He presented evidence of moderate rickets with very low serum calcium and high inorganic phosphorus. During periods A B and C

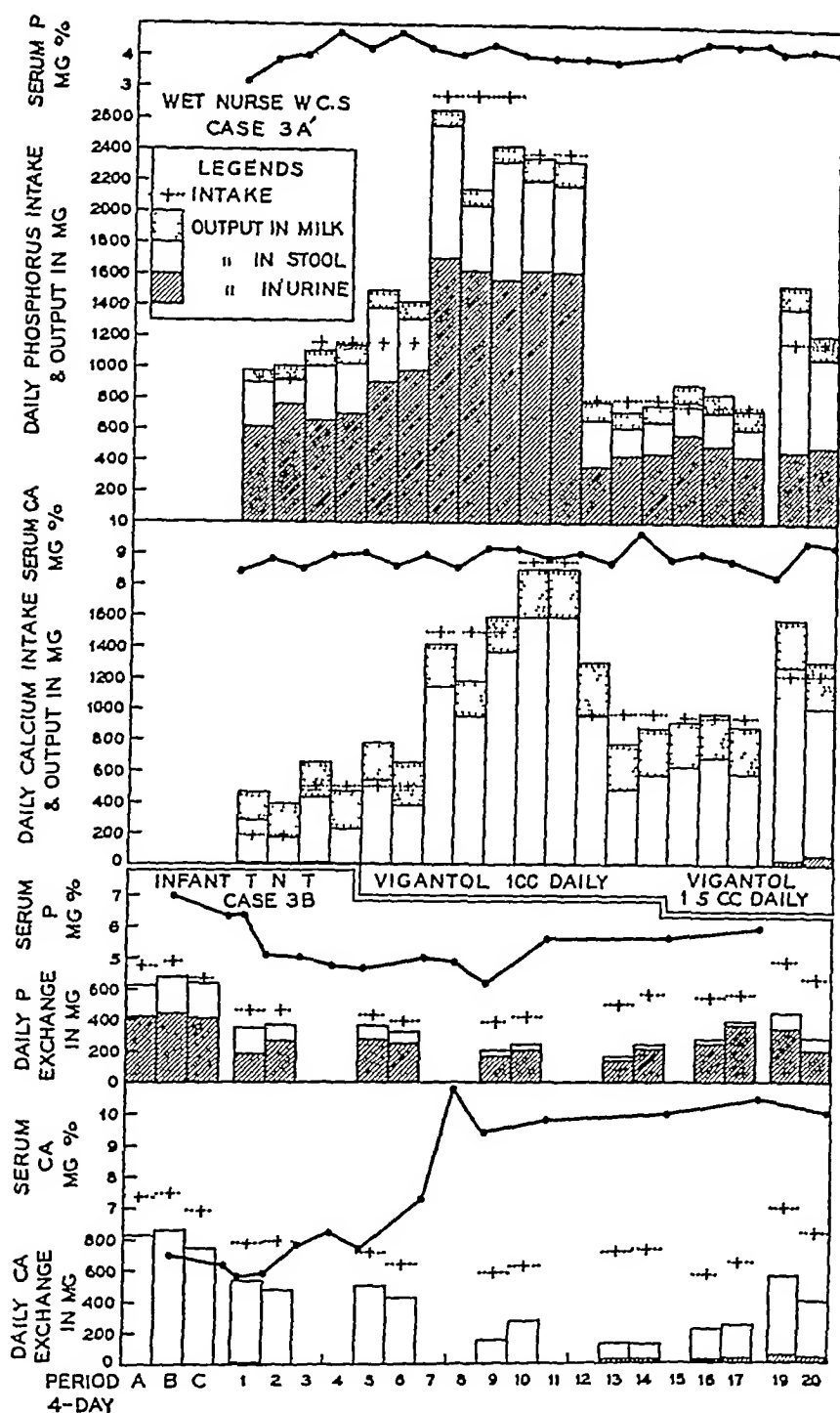


FIG 3 CALCIUM AND PHOSPHORUS METABOLISM OF CASES 3A' AND 3B

The wet nurse was presumably normal, although the Vigantol given probably helped her in maintaining mineral balance. The infant with rickets and tetany did poorly on Kilm and on breast milk of the wet nurse until vitamin D was given to the latter. Then remarkably good mineral retention occurred in the infant.

TABLE VII

Wet nurse W C S Case 3a' Calcium, phosphorus and nitrogen metabolism of a presumably normal lactating woman before and after vitamin D therapy

Date 1933	Period four day	Calcium, average per day					Phosphorus, average per day					Nitrogen, average per day					Serum	
		In-take	Urine	Stool	Milk	Balance	In-take	Urine	Stool	Milk	Balance	In-take	Urine	Stool	Milk	Balance	Calcium	Phosphorus
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	grams	grams	grams	grams	grams	mgm. per cent	mgm. per cent
I 19-22	1	186	8	278	180	-280	930	616	288	76	-50	12.35	7.69	1.62	1.21	+1.83	8.42	3.15
I 23-26	2	186	5	170	213	-202	930	760	156	89	-75	12.35	7.15	1.32	1.44	+1.54	8.80	3.87
I 27-30	3	505	12	422	223	-152	1157	666	350	93	+48	13.76	8.39	1.59	1.44	+1.34	8.49	4.08
I 31-II 3	4	505	3	218	227	-1	1157	708	331	116	+3	13.76	7.93	1.60	1.64	+1.60	8.94	4.82
II 4-7	5*	505	5	533	247	-280	1157	910	472	111	-336	13.76	7.44	2.01	1.65	+2.66	8.90	4.25
II 8-11	6	505	5	371	280	-151	1157	986	327	111	-267	13.76	8.25	1.66	1.79	+2.06	8.63	4.82
II 12-15	7	1491	4	1144	268	+75	2744	1708	853	108	+75	13.76	8.10	2.28	1.77	+1.61	8.93	4.33
II 16-19	8	1491	5	951	223	+212	2744	1624	428	99	+593	13.76	6.90	2.20	1.58	+2.19	8.54	4.14
II 20-23	9	1491	2	1301	229	+101	2744	1568	762	93	+321	13.76	7.40	2.52	1.59	+2.25	9.18	4.43
II 24-27	10	1951	3	1587	312	+47	3284	1630	585	141	+28	10.73	6.49	1.65	1.80	+0.78	9.15	4.08
II 28-III 3	11	1951	4	1591	303	+52	2384	1620	536	133	+75	10.72	5.84	1.41	1.97	+1.50	8.67	3.99
III 4-7	12	965	1	960	338	-334	797	366	301	122	-8	10.72	7.27	1.38	1.98	+0.09	8.97	3.99
III 8-11	13	965	4	479	293	+189	797	435	181	116	+64	10.72	7.29	0.84	1.78	+0.81	8.69	3.91
III 12-15	14	965	2	574	295	+94	797	452	200	109	+35	10.72	6.68	0.84	1.64	+1.56	9.09	4.28
III 16-19	15	948	2	623	293	+30	725	377	210	112	-174	8.92	7.74	0.98	1.77	-1.57	8.82	4.62
III 20-23	16	948	0	690	284	-26	725	508	214	114	-111	8.92	6.87	1.26	1.83	-1.04	9.01	4.49
III 24-27	17	948	1	592	297	+58	725	436	177	126	+14	8.92	6.12	1.01	1.75	+0.04	8.60	4.16
III 28-31	18																8.20	4.30
IV 1-4	19	1221	28	1249	303	-250	1174	469	937	146	-378	16.25	9.11	2.26	2.51	+2.37	9.41	4.46
IV 5-8	20	1221	60	948	307	-94	1174	501	575	139	-41	16.25	10.45	1.58	2.36	+1.66	9.30	4.36

* Vigantol 1 cc. daily starting from this period on.

TABLE VIII

Infant T N T Case 3b Calcium phosphorus and nitrogen metabolism of a rachitic infant on cow's milk breast milk from the wet nurse and combinations thereof

Date 1937 1938	Period four-day	Milk intake		Calcium, average per day					Phosphorus, average per day					Nitrogen, average per day					Serum		Medication (2)		Body weight
		Breast (1)	Klim (2)	In-take	Urine	Stool	Balance	Retention	In-take	Urine	Stool	Balance	Retention	In-take	Urine	Stool	Balance	Retention	Calcium	Phosphorus	Calcium	Phosphorus	
		cc.	cc.	mgm.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	grams	grams	grams	grams	per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	
XI 19-25	A	68	793	1073	0	826	+316	23	753	418	304	+132	13	4.06	3.66	0.43	+0.98	24			13.74	6.72	
XI 26-30	B	48	823	1092	3	880	+333	21	774	479	213	+122	12	4.44	2.86	0.43	+1.23	30	8.80	7.07		6.97	
XI 31-12 1	C	29	703	933	1	744	+192	31	693	411	338	+55	4	3.83	2.16	0.43	+0.94	27				8.96	
12 2-5	1	420	430	773	9	824	+130	31	400	180	168	+112	34	2.96	1.23	0.54	+1.31	41	4.77	6.40		5.81	
12 6-9	2	420	430	780	3	465	+313	40	404	303	106	+68	30	2.97	1.23	0.40	+0.78	28			7.56	6.12	
12 10-13																						6.10	
12 14-17																						6.14	
12 18-21	5*	430	381	711	0	494	+117	30	432	250	63	+70	16	2.04	1.55	0.35	+1.14	38	5.00	4.61		3.25	
12 22-25	6	430	353	633	1	420	+212	33	431	248	74	+69	14	2.04	1.84	0.38	+0.80	35	7.34	5.02		6.50	
12 26-29	9	350	356	879	1	147	+431	73	393	185	36	+192	49	2.82	1.31	0.35	+0.96	35	10.53	4.88		6.23	
12 30-1 3	10	462	373	824	2	276	+66	66	431	204	41	+174	41	2.84	1.50	0.35	+0.78	29	9.40	4.16		6.35	
1 4-7	11	780	0	718	15	113	+562	83	499	137	23	+234	67	1.79	0.79	0.23	+0.77	42				6.24	
1 8-11	12	780	0	727	17	105	+608	88	566	214	25	+237	58	1.58	0.63	0.27	+0.79	47	10.01	5.56		6.25	
1 12-15	13	820	0	565	18	98	+631	84	544	234	37	+263	48	1.48	0.63	0.28	+0.53	25			480	122	6.28
1 16-19	14	705	0	644	23	125	+499	78	560	371	28	+161	29	1.17	0.86	0.34	+0.67	44			323	127	5.61
1 20-23	15	705	0	644	23	125	+499	78	560	371	28	+161	29	1.17	0.86	0.34	+0.67	44			423	127	6.68
1 24-27	16	644	0	644	37	341	+438	83	663	206	61	+295	43	2.36	1.32	0.27	+1.29	23				6.53	
1 28-31	17	644	0	644	37	341	+438	83	663	206	61	+295	43	2.36	1.32	0.27	+1.29	23				6.73	

(1) The small amounts of breast milk given during periods A, B and C were from the mother (Case 3a) and the breast milk for the rest of the periods was supplied by the wet nurse (Case 3a' Table II)

(2) Klim* used for periods A, B and C was analyzed to contain 132.5 mgm. calcium, 93.9 mgm. phosphorus and 0.492 grams nitrogen per 100 cc. while that used for the remaining periods, 129.2 mgm calcium 94.4 mgm phosphorus and 0.484 grams nitrogen per 100 cc. The formula was made up by dissolving one part of Klim whole milk powder in eight parts of water

(3) Calcium given in periods 1-2 was in the form of 10 per cent gluconate intramuscularly that in periods 5 and 6 3 per cent gluconate by mouth and that in periods 13-17 7.7 per cent lactate by mouth. Phosphorus was given as disodium phosphate solution per os.

* Wet nurse starting to receive vitamin D from this period.

(Figure 3 and Table VIII) while on a Klim formula (with small amounts of breast milk from the mother) providing about 1,000 mgm of calcium, large amounts of calcium came out in the stools, leaving a net retention of only 21 to 23 per cent of the intake. Phosphorus retention was even poorer, being 4 to 18 per cent. Serum calcium went down to as low as 4.77 mgm per cent, whereupon convulsive seizures occurred. Evidently the infant, like the mother, was markedly deficient in vitamin D.

After the manifest tetany was brought under control by means of parenteral calcium therapy, metabolic observations were resumed, and breast feeding from the wet nurse was begun. Periods 1 and 2 served as control for a regimen of "half breast milk and half Klim formula" furnishing from 600 to 800 mgm. of calcium and from 400 to 500 mgm. of phosphorus. On this regimen the retention of both elements was distinctly poor, though slightly better than during periods A to C when Klim constituted almost the sole source of nutrients. This indicates that the wet nurse prior to vitamin D supplements, while capable of maintaining her skeleton intact for a time, was probably unable to secrete milk of sufficiently high antirachitic potency to correct the metabolic defect of the infant.

From period 5 on the wet nurse received vitamin D. The effect of this supplement to the milk provider on the infant's metabolism was not evident during the first 2 periods (periods 5 and 6), but subsequently considerable improvement was noticed. During periods 9 and 10, calcium retention rose to 56 to 73 per cent and phosphorus retention to 41 to 49 per cent. The best performance came during periods 13, 14, 16 and 17, when breast milk from the wet nurse alone was fed with additions of calcium lactate and disodium phosphate to maintain the calcium and phosphorus intake. Here the average calcium retention of the 4 periods amounted to 81 per cent, and the average phosphorus retention to 50 per cent of the intake. The high degree of retention of calcium was brought about entirely by a reduction in the stool elimination, while that of phosphorus was caused by a reduction in both the urine and stool excretion.

The last 2 periods were devoted to a study of the effects of feeding Klim alone comparable to the regimen in periods A to C. While vitamin D was still operative, as it was presumably so during periods 19 and 20, both calcium and phosphorus retention were decidedly better than during periods A to C when marked vitamin D deficiency had existed. However, the degree of retention was not as good as during the exclusive breast-feeding periods, possibly suggesting certain peculiarities in the cow's milk that rendered its mineral contents less readily absorbable.

The behavior of serum calcium is worth noting. After it reached its lowest ebb with the onset of convulsions, parenteral calcium was capable of raising it only just enough to stop the convulsions, but with the feeding of

"vitaminized" breast milk it rose precipitously to normal in the course of twelve days, and remained so throughout the balance of the experiment. Serum inorganic phosphorus, high to start with, went down to below normal when serum calcium reached the normal level, and subsequently it also rose to normal.

Coinciding with the correction of the biochemical abnormalities, there was clinical improvement as well as roentgenologic evidence of healing of the rachitic bone changes in the course of a little over two months.

Case 4a Mother W H S This patient had active osteomalacia with slightly low serum calcium and markedly low serum inorganic phosphorus. She was admitted at the eighth month of pregnancy, and metabolic observation during the latter part of gestation showed that even balances were obtained on high levels of calcium intake and that the administration of Vigantol, 5 cc daily for four days, was followed by progressive and marked retention of calcium and phosphorus, and a return of serum calcium and inorganic phosphorus to normal. The Vigantol given for the above-stated period, together with cod liver oil in 30 cc. daily doses for six days immediately postpartum, constituted the only supply of vitamin D prior to the studies on lactation.

As shown in Figure 4 and Table IX, metabolic observations, begun seventeen days postpartum, were continued for 26 four-day periods on intakes of calcium ranging from 1,407 to 1,656 mgm per day. No vitamin D was supplied until the 19th period when Vigantol was started in daily doses of 2 cc. or 24,000 international units and continued for the remainder of the experiment. During the first 18 periods without vitamin D, the calcium balances showed considerable fluctuation. However, the trend is one of progressive loss of calcium from the body. If the 18 periods are divided into 3 series of 6 periods each, and the balances in each series averaged, one obtains a daily calcium balance of +9 mgm, -19 mgm. and -70 mgm. for periods 1 to 6, 7 to 12 and 13 to 18, respectively. This progressive weakening of calcium balance indicates most probably that the limited amount of vitamin D received prior to the studies was gradually being depleted in the course of three or four months. Another sign of vitamin D depletion, or rather deficient intestinal absorption, is the diminution and disappearance of urinary calcium with a correspondingly increased stool calcium.

After vitamin D therapy (beginning with period 19), the situation was entirely reversed. Stool calcium gradually decreased while urinary calcium returned. But the conservation through the bowel was much greater in extent than the loss through the urinary tract and, as a result, the balance became increasingly positive. The average daily calcium retention for the 8 periods amounted to 133 mgm. Thus vitamin D administration during lactation in a woman with skeletal decalcification, similar to Case 2a, was capable of conserving the metabolism of calcium to such an extent as to enable her not only to meet the strenuous requirements of lactation, but

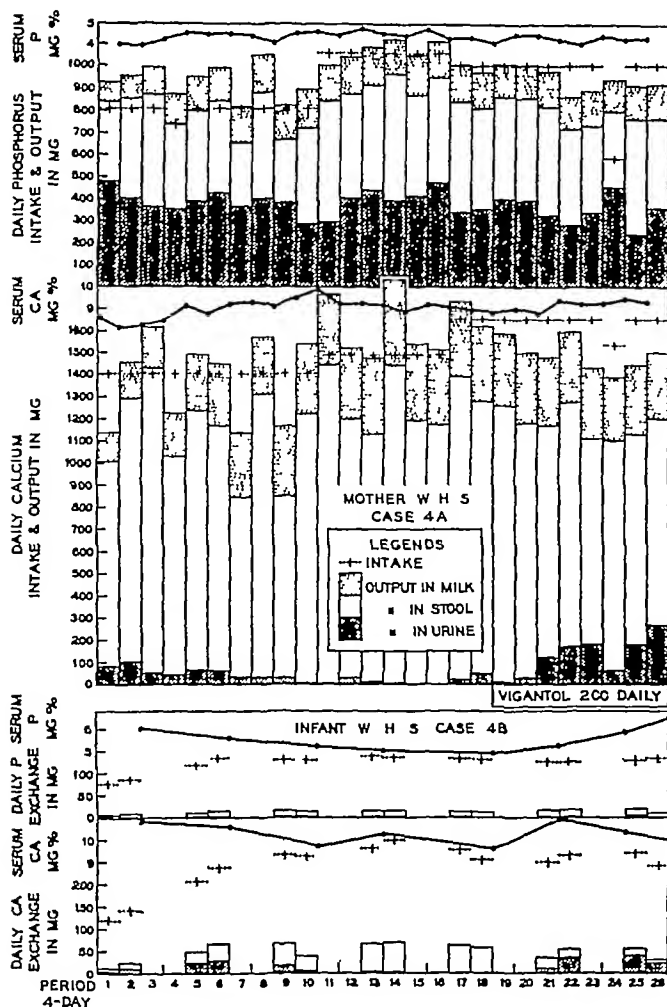


FIG. 4 CALCIUM AND PHOSPHORUS METABOLISM OF CASES 4A AND 4B

The mother with osteomalacia had a limited amount of vitamin D prior to study but showed increasing mineral loss as observation proceeded until Vigantol was given. The infant exhibited good retention of calcium and phosphorus throughout, but a decrease, and later disappearance of urinary calcium with corresponding increase of fecal calcium occurred while the mother was being depleted of vitamin D. Reinstitution of vitamin D therapy in the mother brought about a reversal in the partition of calcium between urinary and fecal elimination.

TABLE IX

Mother W H S Case 4a Calcium, phosphorus and nitrogen metabolism in a case of osteomalacia during lactation at various states of vitamin D nutrition

Date 1938	Four-day period	Calcium average per day					Phosphorus average per day					Nitrogen average per day					Serum		
		In-take	Urine	Stool	Milk	Bal-ance	In-take	Urine	Stool	Milk	Bal-ance	In-take	Urine	Stool	Milk	Bal-ance	Cal-cium	Phos-phorus	Phos-pha-tase
		mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	grams	grams	grams	grams	grams	mgm per cent	mgm per cent	units
II24-27	1	1407	83	924	128	+272	812	480	360	82	-110	10.61	6.70	1.34	1.19	+1.38	8.64	5.97	
II28-III3	2	1407	103	1191	162	-49	812	405	452	98	-143	10.61	6.10	1.60	1.45	+1.46	8.16	4.00	
III 4-7	3	1407	51	1378	189	-211	812	364	507	121	-180	10.61	6.15	1.81	1.60	+1.05	8.23	3.97	3.61
III 8-11	4	1409	45	982	209	+174	743	352	389	133	-131	9.95	5.69	1.20	1.77	+1.29	8.44	4.22	
III12-15	5	1407	67	1167	261	-88	812	390	418	142	-138	10.61	5.72	1.41	1.86	+1.62	9.10	4.56	
III16-19	6	1407	60	1108	282	-43	812	426	417	159	-190	10.61	5.72	1.10	1.97	+1.82	8.74	4.48	
III20-23	7	1407	34	812	287	+274	812	364	290	159	-1	10.61	5.79	0.89	2.01	+1.92	9.20	4.50	2.76
III24-27	8	1407	33	1280	281	-187	812	401	481	167	-237	10.61	5.77	1.27	2.00	+1.57	9.31	4.40	
III28-31	9	1407	31	819	316	+241	812	381	287	154	-10	10.61	7.63	0.64	2.05	+0.29	9.08	4.04	
IV 1-4	10	1407	6	1218	318	-135	812	287	433	157	-65	10.61	6.72	1.24	2.00	+0.65	9.48	4.53	
IV 5-8	11	1492	4	1443	319	-274	1065	294	553	161	+57	12.96	7.19	1.75	1.96	+2.06	9.80	4.64	3.28
IV 9-12	12	1492	31	1168	323	-30	1065	402	474	165	+24	12.96	6.86	1.53	2.04	+2.53	9.24	4.48	
IV13-16	13	1492	11	1120	348	+13	1065	441	574	173	-23	12.96	8.76	1.36	2.07	+0.77	9.24	4.78	
IV17-20	14	1492	6	1478	348	-340	1065	396	570	159	-60	12.96	7.40	1.55	2.00	+2.01	9.20	4.53	
IV21-24	15	1492	3	1192	348	-51	1065	413	459	176	+17	12.96	8.10	1.42	1.99	+1.45	8.93	4.22	2.24
IV25-28	16	1492	0	1176	340	-24	1065	475	474	161	+35	12.96	9.90	1.45	1.98	-0.37	9.19	4.72	
IV29-V2	17	1656	21	1374	341	-80	1006	340	502	165	-1	14.84	8.40	1.78	1.97	+2.69	9.10	4.27	
V 3-6	18	1656	48	1233	321	+63	1006	354	458	160	+34	14.84	8.14	1.86	1.85	+2.99	8.84	4.39	
V 7-10	19*	1656	13	1250	326	+67	1006	401	466	152	-13	14.84	8.81	1.99	1.97	+2.07	8.84	4.07	
V11-14	20	1656	31	1151	320	+154	1006	396	463	153	-6	14.84	8.94	1.75	1.99	+2.16	9.02	4.54	
V15-18	21	1656	124	1048	310	+174	1006	328	494	156	+28	14.84	8.92	2.28	2.09	+1.55	8.80	4.44	
V19-22	22	1656	173	1105	323	+55	1006	284	428	153	+241	14.84	8.24	1.86	2.01	+2.73	9.38	4.24	2.86
V23-26	23	1656	182	933	329	+112	1006	339	398	160	+109	14.84	8.62	1.72	2.10	+2.40	9.19	4.00	
V27-30	24	1537	62	1042	287	+146	587	457	342	143	-355	8.42	6.43	1.08	1.81	-0.90	9.24	4.43	1.85
V31-V13	25	1656	180	954	314	+208	1006	241	425	149	+191	14.84	7.89	1.57	1.97	+3.41	9.44	4.20	
V14-7	26	1656	268	943	295	+150	1006	363	401	163	+79	14.84	8.38	1.61	1.90	+2.95	9.31	4.35	2.18

* Vigantol 2 cc daily starting from this period

also to retain considerable amounts of the mineral for the replenishment of the skeletal store

Phosphorus balances were mainly negative, especially during periods 1 to 10 when the intake was relatively low. But after vitamin D therapy substantial amounts of phosphorus were retained. Serum calcium and inorganic phosphorus were maintained at fairly steady levels.

Case 4b Infant W H S This was a normal baby. Although his mother (Case 4a) had active osteomalacia during pregnancy, the vitamin D given during the latter part of gestation, albeit limited in amount, might have contributed to the relative wellbeing of the infant from the standpoint of mineral metabolism. While exclusively fed on the mother's milk, the calcium, phosphorus and nitrogen balances of the infant were studied *pari passu* with those of the mother, although with him 2 periods of actual study were alternated with 2 "rest" periods. Comparable to the normal infant (Case 1b) and the 2 rachitic infants (Cases 2b and 3b) following feeding with breast milk "vitaminized" via the mother, this infant showed such avidity in retaining calcium and phosphorus that he left only small amounts of these elements to be eliminated in the urine and stool. As shown in Figure 4 and Table X approximately 80 per cent of the calcium and 90 per cent of the phosphorus in the milk were retained.

As the mother was being depleted of vitamin D, the infant began to show a slight decrease in calcium retention (periods 13 to 18). What is more striking is

the behavior of the urinary and stool calcium. As impoverishment of vitamin D proceeded in the mother, there was in the infant a progressive decrease in the urinary calcium to the point of disappearance, coinciding with a steady increase in the stool calcium.

This phenomenon was interpreted as an early sign of vitamin D deficiency, because when the mother began to be replenished with vitamin D, the infant showed a return of urinary calcium with a corresponding decrease of stool calcium. The net gain of calcium was somewhat better during the last 4 periods (periods 21 to 22 and 25 to 26) of observation when the mother was receiving Vigantol.

It is of interest to note that throughout the entire 26 periods, the phosphorus retention was maintained at the uniform high level of 90 per cent with very little variation in the partition of phosphorus between the urine and the stool, suggesting that phosphorus metabolism was possibly a less sensitive index of vitamin D nutrition than calcium metabolism.

Both the serum calcium and inorganic phosphorus were maintained within normal limits throughout the periods of observation. However, there seemed to be a decline in both elements toward the end of the depletion period in the mother, with a return to the initial levels subsequently when the mother was being replenished with vitamin D.

Roentgenograms of the wrists taken periodically revealed that the epiphyseal lines, normal to start with, began to be fuzzy with "hopping" at the end of the period of

depletion in the mother. After the infant had received for a month breast milk from the mother being given vitamin D x ray showed improvement in what were considered to be the early changes of rickets.

DISCUSSION

Observations made simultaneously on the calcium, phosphorus and nitrogen metabolism of the lactating mother and of the breast milk-fed infant of the type recorded in the present communication have not been reported in the literature as far as we are aware. Yet such experiments are of importance in throwing light on the nature and extent of the relationship between the maternal and infantile nutrition by way of the breast milk. From the results presented several points of interest may be mentioned.

Maternal metabolism during lactation. It is generally accepted that considerable difficulty exists in maintaining women in mineral balance during lactation. Hunscher (10) and Hummel and associates (9) failed to maintain actively nursing women in calcium balance on very high intake (3 to 4 grams per day) during the early part of lactation. Only during late lactation, when milk flow diminished, was calcium stored (5). Supplementing the usual home diets with cod liver oil although improving the calcium balance, did not always change a negative to a positive balance (15). Garry and Stiven (6) who have reviewed the recent literature on dietary re-

quirements in pregnancy and lactation, recommend 1 to 2 grams of calcium for milk yields of from 500 to 1000 cc. and calcium contents from 20 to 30 mgm. per 100 cc. milk.

To insure the proper utilization of the above-recommended amount of calcium intake, vitamin D must be added. Thus all the 4 lactating women studied maintained themselves in calcium balance on intakes between 1 to 2 grams when vitamin D was operative. These data are especially significant when it is remembered that they were obtained from women in early lactation with milk yields exceeding one liter a day and calcium content more than 25 mgm per 100 cc. On the other hand when vitamin D was deficient (Case 2a) or being depleted (Case 4a), negative balance prevailed. Therefore, some of the reported difficulties in maintaining calcium balance in lactation on relatively high intake can be as justly attributed to vitamin D undernutrition as to the heightened requirements of this phase of the reproductive cycle.

To the woman with prior vitamin D and calcium deficiency as in osteomalacia the administration of vitamin D has the added importance of making it possible for her to store calcium for her skeletal replenishment in the face of extra drain of lactation. This is amply illustrated by the data obtained from Cases 2a and 4a.

The situation in late lactation, when the milk flow diminishes, may be less stringent. Of the

TABLE X

Infant W H S Case 4b Calcium, phosphorus and nitrogen metabolism of a presumably normal breast-fed infant showing changes due to the varying state of vitamin D nutrition in the mother

Date 1930	Period for day	Breast milk intake	Calcium, average per day					Phosphorus, average per day					Nitrogen, average per day					Boron			Body weight
			Intake	Urine	Stool	Balance	Retention	Intake	Urine	Stool	Balance	Retention	Intake	Urine	Stool	Balance	Retention	Calcium	Phosphorus	Phosphate	
		cc.	mgm.	mgm.	mgm.	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	per cent	grams	grams	grams	grams	per cent	mgm. per cent	mgm. per cent	units	lbsm.
1124-27	1	456	120	4	8	+106	90	77	0	6	+ 71	32	1.07	0.35	0.17	+0.55	51	10.28	6.03	8.31	3.01
1112-1113	2	410	142	12	18	+118	83	89	1	9	+ 78	38	1.10	0.38	0.20	+0.17	14	10.88			3.06
1113-115	3	720	217	23	25	+160	79	118	2	5	+108	92	1.55	0.54	0.21	+0.79	51	10.71			2.67
1116-19	4	780	237	29	36	+170	79	134	3	13	+119	89	1.66	0.65	0.35	+0.53	41	10.58	5.56	17.00	2.70
1118-21	5	900	256	17	50	+190	75	129	3	18	+112	95	1.78	0.63	0.34	+0.87	50	10.70			4.15
1121-1	10	900	263	6	32	+225	80	121	3	11	+118	80	1.67	0.61	0.19	+0.87	82	8.70	5.18	18.42	4.40
1118-13	12	916	280	3	64	+213	78	120	1	14	+124	60	1.87	0.62	0.24	+0.81	48				4.23
1117-25	14	1020	300	1	67	+232	77	137	1	14	+122	60	1.72	0.62	0.22	+0.88	81	10.75	8.00		4.97
1125-13	17	1020	277	1	63	+214	77	124	0	12	+121	60	1.56	0.67	0.34	+0.75	48	8.85	4.85		4.67
V 3-6	18	1020	255	0	87	+168	78	121	1	11	+119	81	1.51	0.57	0.17	+0.77	51				4.76
V15-19	21	1020	240	9	25	+115	84	126	1	16	+108	64	1.53	0.47	0.28	+0.56	81	11.37	5.24	5.08	4.74
V19-23	22	1020	266	34	19	+313	80	126	3	12	+119	68	1.65	0.55	0.35	+0.53	41	10.34	8.37	13.93	4.20
V21-V23	23	1060	271	30	18	+217	80	129	3	14	+110	62	1.70	0.67	0.24	+0.79	48				4.80
V1 4-7	26	1060	243	30	7	+206	85	125	3	10	+123	91	1.57	0.58	0.14	+0.53	56	10.01	6.49	10.60	4.77

* Mother starting to receive Vigantol 2 cc. per day right after the completion of this period

TABLE XI

Data selected and averaged from Tables IV, VI, VIII and X to show the calcium retention of the infants in the present study for comparison with the maximum recorded retention of breast fed infants compiled from the literature by Leitch (11)

Age	Leitch				Case 1b				Case 2b				Case 3b				Case 4b			
	In-take	Bal-ance	Re-ten-tion	Ideal retention	Pe-riod	In-take	Bal-ance	Re-ten-tion	Pe-riod	In-take	Bal-ance	Re-ten-tion	Pe-riod	In-take	Bal-ance	Re-ten-tion	Pe-riod	In-take	Bal-ance	Re-ten-tion
months	mgm	mgm	per cent	mgm		mgm	mgm	per cent		mgm	mgm	per cent		mgm	mgm	per cent		mgm	mgm	per cent
0-1	208	82	39	130	3-4	290	220	76												
1-2	127	85	67	310	5-9	336	255	76									1-2	131	113	86
2-3	149	102	68	250													5-10	246	191	78
3-4	235	109	46	240					8-12	266	207	78					13-18*	278	228	82
4-5	236	142	60	220													21-26	257	213	83
5-6	221	65	29	170									13-17	663†	535	81				

* Beginning vitamin D deficiency may be present during these periods

† Intake raised by the addition of calcium lactate

5 lactating women reported on in a previous paper (16), 4 were in relatively late lactation with milk yields ranging from 280 to 460 cc per day, and it was possible for them to store calcium on intakes varying from 400 to 700 mgm. However, this was possible only after the exhibition of vitamin D.

Metabolism of breast-fed infants In 1937 Leitch (11) assembled all available recent data on calcium metabolism of normal infants fed exclusively on breast milk. The maximum recorded retention for the entire series was shown for each monthly period from the first to the sixth month. This ranged from 65 to 142 mgm per day or from 29 to 68 per cent of the intake. In each instance the amount of calcium retained fell very much short of the requirement of 170 to 310 mgm calculated for skeletal growth to maintain the calcium content of the body at 10 grams per kgm. Should such a state prevail, a skeleton of more inferior grade of calcification than that at birth would be built up, leading to rickets and osteoporosis. Leitch finds it difficult to reconcile his calculation with the anomalous situation where feeding of breast milk, the natural food for the infant, would lead to deterioration of the skeleton.

A review of the data on the 4 infants included in the present work (rearranged in Table XI) suggests that such an anomalous position need not arise if milk intake is adequate and if vitamin D is operative. When such was the case, each of the 4 infants showed an average daily retention approaching the ideal calculated by Leitch and exceeding the maximum retention recorded in the

literature. The explanation for such excellent retention in our infants is probably two-fold. First, the quantities of breast milk consumed were sufficient to provide a total intake of calcium to meet the requirement. Second, by virtue of the conserving and absorption-promoting action of vitamin D, the retention of calcium became exceedingly efficient, from 76 to 86 per cent of the intake being retained. It is true that 2 of the infants (Cases 2b and 3b) had rickets in which vitamin D treatment should give rise to extraordinary retention to restore the depleted skeletal store. However, the other 2 infants, who had no rickets to start with, exhibited the same high degree of calcium retention, suggesting that breast feeding in adequate amounts, fortified by vitamin D, would lead to normal skeletal growth of the infant.

Vitamin D in human milk The position of the vitamin D content of human milk is rather vague. Hess (8) has demonstrated that, whereas 25 cc of ordinary human milk daily failed to cure rickets in rats, the same could be made effective by treating the mother with ultraviolet irradiation. Out-house, Macy and Brekke (22) reported that pooled milk from wet nurses on an average American dietary in amounts of 25, 30, or 40 cc daily contained no antirachitic factor as tested on rachitic rats, although 30 cc of cow's milk fed daily induced marked healing. Bunker, Harris and Eustis (2) found that milk of human mothers fed previously on "vitamin D" milk of the cow was potent in the antirachitic factor for the rat.

When the antirachitic potency of breast milk

from mothers receiving vitamin D supplements is tested on the infant, it is generally believed that some though uncertain degree of protection is transferable to the suckling children. Weech (24) in a survey of 47 infants breast fed by mothers given cod liver oil, found that the degree of intensity of the rachitic process as judged by roentgenograms of wrists and the product of serum calcium and phosphorus was inversely proportional to the amount of cod liver oil administered. Even in the group of 4 women receiving the largest amount of cod liver oil (50 to 60 ounces in six months) 2 of the infants had x ray evidence of rickets. Barnes, Cope, Hunscher and Brekke (1) studied a woman whose diet was superior in quality and, in addition was fortified with 2 quarts of cow's milk daily in which 300 units of a vitamin D concentrate was incorporated. It was found that the milk secreted was not sufficiently enriched by vitamin D to heal rickets in 3 colored infants or in experimental rachitic rats. Her own breast fed baby, however, showed no signs of rickets throughout the investigation.

From the observations presented in this paper there is no doubt that the administration of vitamin D to the mother in ordinary therapeutic doses (12,000 to 24,000 international units) is capable of sufficiently enriching her milk by vitamin D to prevent (Cases 1b and 4b) or cure (Cases 2b and 3b) rickets in the infant. When vitamin D is withheld from the mother for a period of three months or so, as in Case 4a, her milk will be sufficiently impoverished to produce early evidence of vitamin D deficiency in the infant. This demonstrates in a somewhat novel way the dependence of the infant on the mother from the standpoint of vitamin D nutrition via breast milk.

Importance of vitamin D supplement to the mother during lactation. It is usually recommended that infants be started with vitamin D supplements in the early months of life, while nursing mothers receive relatively scant attention in this respect. This work should give credence to the view that it is just as important to supplement the mother as the child, if not more so. Vitamin D administration to the lactating mother is necessary to maintain her in balance in case her skeletal store is normal and to enable her to store calcium if she has prior skeletal depletion as in osteomalacia. In adequate doses the administra-

tion of vitamin D to the mother would supply her milk with antirachitic properties sufficient for the care of the child.

The minimal dosage for the mother that would be efficacious both for the mother and for the infant is not determined. The daily dosage of 12-000 to 24,000 international units is probably more than the minimal effective dose. Nor is there evidence to indicate that such dosage need be kept up uninterruptedly throughout the nursing period. However to render a relatively small dosage of vitamin D effective it is important that the level of calcium intake be raised over the ordinary requirement. The recommended amount of 1 to 2 grams of calcium per day will likely be sufficient for the purpose.

SUMMARY

1 Data on calcium phosphorus and nitrogen metabolism were obtained on 4 women while they were supplying breast milk to 4 infants from whom similar data were secured at the same time, showing intimate relationship in the state of vitamin D nutrition between the mother and the infant during the nursing period.

2 In the first set of cases the mother had healing osteomalacia with good retention of calcium and abundant store of vitamin D during gestation, and the infant was born normal. During lactation the mother maintained herself in mineral balance on relatively high intake, while the infant retained the major portion of the calcium and phosphorus intake in the milk.

3 The second pair of cases consisted of a mother with osteomalacia and tetany and her infant with rickets and tetany. Both showed poor retention of calcium and phosphorus but after vitamin D administration to the mother, the metabolic defects of the mother as well as the infant were corrected. The mother, as a result of vitamin D therapy was able to absorb sufficient minerals not only for the heightened requirements of lactation but also for the reparation of her depleted skeletal store. The infant after receiving the 'vitaminized' milk, showed markedly improved mineral retention.

4 The third experiment was on a presumably normal wet nurse supplying breast milk to a rachitic infant born of an osteomalacic mother. The wet nurse had to have her calcium intake

raised to 15 to 20 grams before she was able to maintain balance. The vitamin D given subsequently probably contributed to this favorable state of affairs. The infant showed poor calcium and phosphorus retention while on a Klim formula and on breast milk prior to vitamin D administration to the wet nurse, but after such supplement, the infant was very much improved in mineral retention as well as in the rachitic bone changes.

5 The last series of observations was made on an osteomalacic mother and her infant. The mother had a limited period of vitamin D therapy during gestation with sufficient improvement in her metabolic disorder to give birth to a normal baby. During lactation while vitamin D was being withheld, she began to show negative mineral balance on relatively high intake, and subsequently, when vitamin D therapy was reinstituted, good retention of calcium and phosphorus. The infant exhibited excellent mineral retention throughout the period of study, but as the mother was being depleted of vitamin D, the urinary calcium excretion diminished and disappeared, corresponding with an increase in the stool. This phenomenon was interpreted as an early sign of vitamin D deficiency, for subsequent supply of vitamin D to the mother induced a reversal in the partition of calcium elimination between the urine and stool in favor of the former.

CASE ABSTRACTS

Case 1a. Mother, Mrs Y W L (Hospital Number 28572), a Chinese woman of 42 was first seen in October 1933 for pain in the bones and debility of three years' duration. Her detailed history was reported in paper II (12) of this series. Briefly stated, she presented a case of advanced osteomalacia with marked skeletal decalcification, deformities and fractures dating back to July 1930 when she gave birth to her fourth child. Her serum calcium was 8.8 and inorganic phosphorus 2.2 mgm per 100 cc., findings that justify the classification of her case as one of low-phosphorus osteomalacia.

She went through detailed metabolic studies from November 1933 to June 1934. Her dramatic response to vitamin D therapy from the symptomatic, roentgenologic and metabolic standpoints was given in paper II (12) and her behavior toward varying levels and ratios of calcium to phosphorus intake was presented in paper III (13).

In September 1934 the patient was readmitted with a slight recurrence of the pain in the bones and difficulty in walking. Her appetite was poor, with occasional nausea and vomiting. She was found to be pregnant

with the expected date of confinement on April 15, 1935. Although slight tenderness was present over the lumbar spine and ribs, roentgenologic examination of the skeleton showed no evidence of exacerbation in osteomalacia. Serum calcium was 8.92 and inorganic phosphorus 2.82. There was minimal pulmonary tuberculosis at right upper lung. A slight degree of anemia developed shortly after admission. Hemoglobin remained at 10 grams, and erythrocyte count at 3.2 millions, in spite of the administration for prolonged periods of ferric ammonium citrate and hydrochloric acid by mouth, and liver extract intramuscularly. Her calcium, phosphorus and nitrogen metabolism was studied throughout pregnancy. From December 1934 to April 1935 her calcium intake was approximately 2 grams per day, and vitamin D in the form of Vigantol was given in doses of from 1 to 3 cc. a day (1 cc. is equivalent to 12,000 international units of vitamin D). During this period of four months, 40 to 50 per cent of the intake of calcium was retained. This would enable her not only to meet the fetal requirements, but also to replenish her own skeletal store of calcium.

The course of gestation was fairly smooth except for an attack of abdominal pain in the latter part of the pregnancy. In view of the pelvic deformities, a cesarean section was performed by Dr J P Maxwell on April 13, 1935, and a normal male baby (weighing 2,885 grams) was delivered. The postoperative course was satisfactory. The patient was studied again from the viewpoint of lactation. The studies began eighteen days *postpartum*, and were continued for 11 four-day periods. This part of the studies, together with observations on other patients during lactation, was reported in Paper VI (14).

The patient was discharged on June 15, 1935 in good condition. She remained well and nursed her baby until September 1936 when she began to have diarrhea, abdominal distension and swelling of legs. On reentry into the hospital in November, although her diarrhea subsided, her general condition deteriorated considerably. There was anasarca with ascites and enlarged liver. Plasma proteins were 1.31 per cent albumin and 2.72 per cent globulin. The pulmonary tuberculosis at right upper lung showed extension. In the right breast there was a firm mass measuring 10 × 8 × 5 cm. associated with enlarged axillary lymph nodes. There had been a firm nodule in her breast for eight years, showing no tendency to grow until four months prior to admission when rapid enlargement commenced. Biopsy of the tumor and axillary lymph gland revealed carcinoma in both, and the section of the sediment of the ascitic fluid withdrawn also exhibited malignant tumor cells. As radical operation was not considered advisable in view of the advanced stage of the disease and of the patient's poor general condition, a course of intensive deep radiotherapy was given to the breast and axilla. Although the tumor masses diminished in size, the general condition of the patient went downhill. Death took place at home in February 1937.

Case 1b. Infant, Y W L (Hospital Number 49062),

was the fifth child of the mother described above. Although the mother had osteomalacia, this was largely healed, and she received large amounts of calcium and vitamin D during gestation. The child delivered at term on April 13 1935 by cesarean section weighed 2,885 grams and measured 53 cm. in length. He was normal in every respect. There was no clinical evidence of rickets. Cord blood serum calcium was 111 mgm. per cent. Roentgenologic examination of the bones of the upper and lower extremities showed normal size, contour and density. He was fed exclusively on mother's milk. Metabolic studies commenced on the twenty-sixth day after birth and proceeded smoothly for 7 four-day periods. General condition remained excellent and weight gain was uninterrupted. On discharge on June 12, he weighed 5,600 grams almost doubling birth weight.

Case 2a. Mother Mrs C S Y (Hospital Number 58397) age 31 was admitted on April 4 1937 for general bodily ache, tingling and spasm of extremities for a year and a half. In December 1934 she gave birth to her first baby which was breast fed until June 1936, when it died. In November 1935 while lactating, she began to have bony aches, followed by numbness and spasticity of the limbs. Active tetanic attacks occurred usually after exposure to cold or prolonged pressure on the limbs. The condition cleared up in April 1936 when she became pregnant for the second time. The bony aches and numbness began to recur in October and became progressively more debilitating. The second child was delivered spontaneously in February 1937. Spastic attacks returned after parturition.

After the birth of the second child, bowels were constipated and bleeding from rectum was frequently noticed on defecation. There was an attack of dysentery in September 1936. Diet consisted of corn, millet, wheat flour rice and vegetables in season. Meat and eggs were very rarely eaten.

On admission the patient appeared well developed, fairly well nourished and not in acute distress. Temperature 37.4 C., pulse 88, blood pressure 110/70 weight 48 kgm. Breasts were lactating with marked venous engorgement. Spleen was just palpable. Hemorrhoids, both external and internal were present and bled on digital examination. There were no skeletal deformities, although pain was complained of in the shoulders, lumbar spine and thighs on motion. The patella tendon reflexes were hyperactive and both Chvostek's and Trousseau's signs were positive. Urine was normal except for the presence of sugar which proved to be lactose. Blood picture was not remarkable. Serum calcium was 7.46 and inorganic phosphorus 4.70 mgm. per 100 cc. phosphatase was 12.9 Bodansky units. On x ray examination slight osteoporosis was present in all the visualized bones but no deformities.

Metabolic studies were started on April 6. The patient developed dysentery with 7 to 10 blood and mucus containing stools a day. Low grade fever was present. Tetany at times became manifest, requiring calcium gluconate intramuscularly for relief. *B. dysenteriae* manife-fermenting group, was isolated from the stools.

Fortunately the attack subsided in six days and was not sufficiently serious to interrupt the metabolic regime. After 3 control periods Vigantol 1 cc. daily was given, and marked symptomatic improvement in the bony aches, numbness and spasticity was noticed.

After 6 periods of metabolic studies there was a recurrence of dysentery necessitating suspension of the rigid quantitative regime. While the administration of Vigantol and high calcium intake were maintained, a semi liquid diet and doses of sodium sulphate were given. In twelve days the bowel condition returned to normal. Metabolic studies were then resumed for 3 periods before the patient was discharged in good condition on May 25 1937.

Case 2b. Infant C C C (Hospital Number 58396) a male baby aged 57 days, was admitted on April 4 1937 for convulsive attacks for four days prior to entry. Although the mother had tetany and osteomalacia as stated above, the birth of the baby was spontaneous and easy. Feeding was exclusively on breasts. The onset of convulsive seizures was sudden without any previous illness. They were generalized with retraction of head, deviation of mouth upward rolling of eyeballs and spastic and clonic contractions of all extremities. Each attack lasted from five to ten minutes.

Examination showed good development and nutrition. Although no convulsions were noticed, both hands and feet were held in spasm and Trousseau's and Chvostek's signs were present. The anterior fontanelle was wide open but craniothoracic rosary and enlargement of the wrists were absent. Liver and spleen were palpable and hydrocele of tunica vaginalis was noted on both sides.

Urine and blood count were normal and stools contained some mucus. Blood serum calcium was 7.8 and phosphorus 8.0 mgm. per cent. phosphatase was 196 units (Bodansky). Plasma proteins were 3.11 per cent albumin and 1.62 per cent globulin. X ray films showed a slight condensation, haziness and lipping at the distal ends of radii, ulnae, tibiae and fibulae. The bones were slightly osteoporotic. These changes were suggestive of mild rickets, but over the lateral aspects of both femurs there was considerable periosteal thickening.

After admission the spastic phenomenon was promptly controlled by doses of calcium gluconate intramuscularly and calcium chloride orally. Metabolic experiments were started at the same time as those on the mother whose milk constituted the sole form of feeding. The studies went on well for 1 period, but during the second and third periods they had to be discontinued on account of a severe local vaccinia reaction to smallpox vaccination, accompanied by fever and return of convulsive attacks. The latter were again controlled by parenteral calcium gluconate and oral calcium chloride, and the whole episode subsided in three or four days. The baby was put on the metabolic regimen again from the fourth period on and satisfactory studies were carried on for 9 more periods.

The infant was discharged in good condition on May 25 1937. Serum calcium and phosphorus were 11.32 and 5.61 mgm. per cent, respectively. X ray examination

repeated on the day of discharge, showed no obvious changes in the bones from that on admission. The weight gain was from 4,900 to 6,100 grams in the course of seven weeks.

Case 3a Mother, Mrs T W T (Hospital Number 61219), a Chinese woman of 38, was admitted on November 29, 1937 with the chief complaint of pain in the lumbar region for thirteen months and in both thighs for five months. She began to have pain in the lower back soon after the onset of her eighth pregnancy thirteen months prior to admission. The pain gradually increased in severity, resulting in difficulty in walking throughout the first and second trimesters of pregnancy. In the latter part of pregnancy the pain also involved the thighs. She was completely bedridden shortly before parturition and has remained so since. Her parturition which took place in August 1937 was slow but spontaneous. The baby (Case 3b) was found to have rickets at the same time that the mother was seen. He was breast fed by the mother but the milk secretion was extremely poor. The patient had attacks of numbness and spasm of hands throughout the course of present illness. She was married at 17, and gave birth to eight children, the eldest one being 20 years of age. She had tingling sensation, spasm of hands and pain in the lower back during her sixth and seventh pregnancies six and four years prior to admission, respectively. Her husband, being a peddler, could scarcely earn enough to feed a family of seven. The diet was extremely inadequate and the patient led a secluded life.

Examination revealed that she was completely disabled in bed, complaining of pain in the muscles and bones whenever she was moved. She had marked muscle spasm and tenderness in the thighs and over the lower ribs. Slight scoliosis and kyphosis of lumbar spine, slight rostration of the symphysis pubis and very narrow pubic arch were present. Signs of Chvostek and Trousseau were positive. Head organs were normal. Thyroid was slightly enlarged. There were no important abnormal findings in the chest and abdomen. Routine laboratory studies, including blood Wassermann test, blood counts, urinalysis and stool examinations were all negative except for a slight transient lactosuria. Fasting blood sugar and non-protein nitrogen were within normal range. Serum calcium was 6.87 and inorganic phosphorus 2.75 mgm. per cent. Plasma proteins were 2.51 per cent albumin and 5.66 per cent globulin. Basal metabolic rate was +21.2 per cent. X-ray examination revealed marked osteoporosis of all the bones, exceedingly marked biconcave deformity of the vertebral bodies, eversion and old fractures of the pubic bones. The right upper lung field appeared slightly clouded.

Metabolic observation extended from December 6, 1937 to April 15, 1938. Her response, clinically and metabolically, to ultraviolet irradiation was reported in paper VII (4) of this series. She gained 9 kgm of weight in four and one-half months, and was discharged on April 16, 1938 much improved symptomatically and roentgenologically.

Case 3a Wet nurse, Mrs W C S (Hospital number

61624), age 23, was admitted on January 14, 1938 as a wet nurse for baby T N T (Case 3b described below). Her past history was irrelevant. She was married a year prior to admission and became pregnant soon afterwards. The course of pregnancy was smooth, and a male child weighing 5 lbs was delivered spontaneously at term on December 17, 1937. The child was fed on her breast soon after birth. The diet for the past year consisted of rice, wheat flour, millet, corn meal and salted and fresh vegetables, but practically no meat or eggs.

Physical examination showed good development and nutrition. Weight 52.2 kgm, height 161 cm. There were no skeletal deformities, bone tenderness, nor signs of tetany. Both breasts were lactating. Except for moderate gingivitis, pharyngitis and endocervicitis, the rest of the physical findings were normal.

Routine urine, blood and stool examinations were normal. Blood Wassermann reaction was negative. Serum calcium and phosphorus were 9.07 and 4.78 mgm per cent, respectively (January 15). Calcium, phosphorus and nitrogen balance studies were started on January 19 and carried out for a total of 22 four-day periods. These included observations on the effects of various levels of calcium and phosphorus intake and of vitamin D therapy. The patient was discharged on April 14 in good condition.

Case 3b Infant, T N T (Hospital Number 61227), male, age three months, was admitted on November 30, 1937 for investigation for possible rickets or tetany on account of advanced osteomalacia in the mother. As mentioned before (Case 3a), this was the eighth child, and his birth was at term and spontaneous but prolonged. As the mother's milk secretion was scanty, breast feeding had to be supplemented by *hsin erh cha* (almond tea), *oufen* (lotus root starch) and *kao kan* (rice flour cake). When two months old the infant had an attack of high fever followed by convulsions lasting for half a day.

Physical development and general nutrition were fair, weight was 5,260 grams. The anterior fontanelle was wide open and the posterior fontanelle partially so. Parietal bosses, Harrison's groove, enlargement of the costochondral junctions and widening of the wrists were noticeable. The head organs were normal except for some nasal discharge and the lungs and heart showed no abnormality. The abdomen was prominent with both spleen and liver palpable at 1 cm. below costal margin.

Urinalysis and stool examinations were normal. Blood showed 10.5 grams hemoglobin, 3.4 million red blood cells, and 11,250 white blood cells with 35 per cent polymorphonuclear leukocytes and 62 per cent lymphocytes. Serum calcium was 5.18 and inorganic phosphorus 5.94 mgm. per 100 cc. and phosphatase was 13.7 Bodansky units. Roentgenograms exhibited moderate osteoporosis of all the bones with slight haziness and cupping of the distal epiphyseal ends of all the long bones of the forearms and legs. Slight beading was observed at the anterior aspects of all the ribs. The diagnosis was moderate rickets (Figure 5). This was likely to be of fetal origin.

For 3 four-day periods (designated as A, B, C, De-

ember 22, 1937 to January 2, 1938 inclusive) while on a formula of "Klim" with a small amount of breast milk from the mother the calcium, phosphorus and nitrogen balances were observed.

On January 18 and 19 attacks of generalized convulsions occurred. Serum calcium and phosphorus were respectively 4.77 and 6.40 mgm. per cent. Calcium ion was 2 mgm. per cent, as determined by the frog heart method (20) and 2.4 mgm. per cent by calculation from total calcium and serum proteins (5.56 grams per cent) according to McLean and Hastings (21). It was considered that the convulsive attacks were a manifestation of tetany. Calcium gluconate (Sandoz) 10 per cent 10 cc. (90 mgm. calcium) was given intramuscularly daily from January 19 to 26 (periods 1 and 2) while the feeding consisted of breast milk from the wet nurse and "Klim," 420 cc. each daily with additions of orange juice.

From January 27 on, calcium gluconate 3 per cent solution was given by mouth in daily doses of 27 cc. (73 mgm. calcium) instead of the 10 per cent solution intramuscularly and the wet nurse received Vigantol 1 cc. (12,000 international units vitamin D) daily. The amount and proportion of "Klim" and breast milk remained approximately the same. While on this regime, balance studies were made on the child for 2 periods from February 4 to 11 (periods 5 and 6).

On February 11 rhinitis developed with purulent discharge from which virulent *B. diphtheriae* were isolated. There was a low-grade fever. Leukocyte count was 10,450 with 52 per cent polymorphonuclears. A total dose of 20,000 units of a concentrated form of diphtheria antitoxin was given intramuscularly in divided doses on February 12 and 13. There was some febrile reaction following the serum treatment. Though the nasal discharge cleared up promptly positive K. L. B. cultures were obtained on several subsequent occasions.

While on essentially the same regime, except for the omission of calcium gluconate by mouth 2 more periods (periods 9 and 10) of metabolic observations were secured from February 20 to 27. From March 8 to 15 (periods 13 and 14) and from March 20 to 27 (periods 16 and 17) feeding was entirely on the breast milk from the wet nurse and the calcium and phosphorus intake was maintained by the addition of a 7.7 per cent solution of calcium lactate 48 cc. daily (480 mgm. calcium) and a solution of disodium phosphate (422 mgm. phosphorus).

The last 2 periods (periods 19 and 20) of metabolic studies were from April 1 to 8 when the feeding was entirely on "Klim," the calcium lactate being omitted.

Just prior to discharge on April 18, 1938 x-ray examination showed evidence of healing of rickets with increased bony density in the course of four months (Fig. ure 6). Body weight on discharge was 6,700 grams.

Case 4a. Mother Mrs. W. H. S. (Hospital Number 65376) age 29 was admitted on December 9 1938 for bony aching particularly of the pelvis and lumbar region with occasional carpopedal spasm for eleven years prior to entry. She was married in 1926 led an indoor life in a cave dwelling in Pingyao, Shansi, and subsisted mainly on cereals and vegetables. Her symptoms, which began

shortly before her first pregnancy in 1927 were considerably aggravated during gestation so that she was confined to bed on account of bone pains and spastic attacks. However the delivery was spontaneous without difficulty and lactation was abundant. In the course of two years the symptoms were gradually improved. On getting up she noticed that her stature dwindled by several inches with knock-knee and pelvic deformities. In 1933 she became pregnant for the second time, and the gestation was terminated by medication at the third month. Her symptoms were aggravated especially during the winter. She was examined in this hospital for the first time in April 1936, when a course of calcium lactate, cod liver oil and ultra violet radiation gave rise to considerable improvement. She returned to Shansi and was pregnant for the third time in March 1937. The backache and leg pains recurred and the pregnancy was artificially aborted at the fourth month. Her fourth pregnancy started in May 1938 and she was admitted during the eighth month of gestation.

Examination showed undernutrition and stunted stature. Weight 44.2 kgm. and height 154 cm. The lower extremities were particularly short in comparison with the trunk and upper extremities. The gait was unsteady with the pelvis tilted from one side to the other in walking while only very short steps could be made. The thighs were strongly adducted and internally rotated, and the knees knocked against each other. The right lower extremity was shorter than the left by 4 cm. Definite tenderness was present over the ribs, spine, pelvis and femora. Chvostek's sign was negative but Trousseau's sign was positive. The heart and lungs were normal. The blood pressure was 80/50. The abdomen was enlarged by the gravid uterus, the fundus of which came up to 28 cm. above the symphysis pubis. Fetal movements and heart sounds were heard. The pelvis was contracted and the transverse outlet measured 7 cm.

Routine urine and stool examinations were normal. Blood count showed 11.2 grams hemoglobin, 3.3 million red blood cells, and 6,500 white blood cells, with 77 per cent polymorphonuclears. Serum calcium was 8.24 and phosphorus 2.77 mgm. per cent, and phosphatase 2.3 Bodansky units. Plasma non protein nitrogen was 21 mgm. per cent, and proteins were 2.97 per cent albumin and 3.24 per cent globulin. Basal metabolic rate was plus 6.7 per cent. Blood Wassermann reaction was negative. Roentgenologic survey of the skeleton showed moderate degree of osteoporosis in all the long bones, slight bilateral concave absorption of the vertebral bodies with irregular curvature of the sacrum, asymmetry of the pelvis, distortion and compression of the head of the femur into the acetabulum and pathological fracture of the right upper femur.

Metabolic studies commenced on December 26 and proceeded for 10 four-day periods prior to the termination of pregnancy. The first 4 periods served as control on high calcium intake alone, and the fifth period was used for the administration of Vigantol in daily doses of 5 cc. (60,000 international units of vitamin D). During the next 5 periods definite progressive increase in cal

cium and phosphorus retention was noticed, with subjective improvement. Serum calcium and phosphorus were 9.46 and 3.86 mgm. per cent, respectively

Cesarean section was performed by Drs H. L. Hsu and S. Lin on February 7, 1939, and a normal male child weighing 2,920 grams was delivered. The puerperium was satisfactory and lactation active. She received 30 cc. cod liver oil daily for six days immediately after parturition.

Metabolic observation on lactation and its effect on the infant was started on February 24, and continued for 26 four-day periods (one hundred and four days). The results are presented in the text. The patient was discharged in excellent condition on June 11, 1939.

Case 4b Infant, W H S (Hospital Number 66085), was a male baby born on February 7, 1939 by cesarean section of an osteomalacic mother who received a limited dose of Vigantol and liberal amounts of calcium during the latter part of pregnancy, as described above. The child weighed 2,920 grams and measured 49.5 cm in length at birth. He was in every way normal. There were no bony deformities and x-ray of the bones showed normal texture with sharp outline and smooth diaphyseal ends (Figure 7). Urine was normal. Blood count showed 9.8 grams hemoglobin, 4.14 million red blood cells, 7,600 white blood cells, with 53 per cent polymorphonuclears. Serum calcium was 10.8 and phosphorus 6.03 mgm. per 100 cc. and phosphatase 8.2 Bodansky units.

Mother's milk constituted the only form of feeding. Metabolic observations were started on February 24, and continued for 2 four-day periods followed by 2 four-day periods without metabolic studies. Thereafter, 2 "observation" periods alternated with 2 "rest" periods. From May 7, that is, after the elapse of 18 periods, the mother received Vigantol 2 cc. (24,000 international units of vitamin D) per day for 8 periods, during which metabolic observations were continued on the infant to ascertain the effect of "vitaminized" milk. Throughout the course of study, the infant took the prescribed feedings quantitatively, remained in excellent condition and gained weight steadily. On discharge on June 11, he weighed 7,000 grams, almost two and one half times the birth weight in the course of four months, although slight anemia persisted.

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CALCIUM AND PHOSPHORUS METABOLISM IN OSTEOMALACIA X. FURTHER STUDIES ON VITAMIN D ACTION EARLY SIGNS OF DEPLETION AND EFFECT OF MINIMAL DOSES

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Hannon and associates (9) in the first communication of this series demonstrated the prolonged beneficial effect of vitamin D on the calcium and phosphorus metabolism in osteomalacia. A daily dose of 7,500 international units of vitamin D in the form of Vigantol (Bayer) administered orally for a period of sixteen days in a case of osteomalacia brought about a marked calcium and phosphorus retention which continued for more than three months after the drug was discontinued. Since the beneficial effect continued unabated even at the end of observation, the actual duration of this action must be longer than three or four months. Similar results were obtained when a series of brief ultraviolet irradiations from a mercury vapor quartz lamp was applied in place of oral administration of vitamin D (3).

In attempting to explain the prolonged action of vitamin D, two possibilities were considered. First, the vitamin D may be stored. This was dismissed at the time because of lack of knowledge on vitamin D storage. The second possibility which was considered the more likely explanation, is related to the vitamin D content of the diets served to the patient. The minimal amounts of vitamin D that may be present in the diets, though insufficient to bring about the reparative process, may suffice to maintain normal calcium and phosphorus metabolism as soon as the initial deficiency is corrected by vitamin D or ultraviolet irradiation therapy.

The present communication is concerned with (1) observation on the antirachitic potency of the vitamin D-deficient diets commonly used in our investigation of calcium and phosphorus metabolism in osteomalacia, (2) the effect of a minimal dose of vitamin D and (3) the changes of calcium and phosphorus metabolism following the withdrawal of vitamin D.

PROCEDURE

Three cases of osteomalacia and one normal newborn baby from an osteomalacic mother were studied in the present investigation. Their clinical histories are briefly described in the appendix. Data on Cases 3 and 4 were published previously in other connections (4, 15). They were kept in the metabolism ward throughout the period of study and quantitative, vitamin D-deficient and low calcium diets were served. The composition of these diets is shown in Table I. Calcium was administered in the form of a 7.7 per cent solution of calcium lactate in Cases 1 and 2. The infant, Case 4, was fed exclusively on measured quantities of his mother's milk. Calcium, phosphorus and nitrogen balances were determined in four-day periods continuously in Cases 1 to 3 but in Case 4, 2 four-day periods of study were alternated with 2 periods without metabolic observations. The organization of the metabolism ward, the preparation of quantitative diets, the collection of excreta and the methods of chemical analysis of food and milk, stool and urine, and serum were described in our early publication (16).

Case 1 was a young woman with osteomalacia who was convalescing from an obstetrical operation and pulmonary tuberculosis in the obstetric service for nearly a month before she was transferred to the metabolism ward. She was put on the basal vitamin D-deficient diet for a long time under metabolic observation just to ascertain the antirachitic potency of the diet. Then acid and alkali salt mixtures of Shohl (17) were given for the purpose of studying the acid and base metabolism which we are not concerned with in the present communication. Then minimal doses of vitamin D in the form of Vigantol and egg were administered on separate occasions to observe their effect on calcium and phosphorus metabolism, particularly the changes following their withdrawal. Case 2 was similarly studied except that prolonged control observation on the basal diet alone was not made. Case 3 was studied primarily from the standpoint of the effect of acid and alkali on the calcium and phosphorus metabolism but the data were reproduced here because they illustrate two of the three propositions in which we are interested in the present communication. Calcium, phosphorus and nitrogen metabolism was observed in Case 4 until what we consider the incipient changes of vitamin D-deficiency appeared. Then the reverse processes were studied after vitamin D was given to the lactating mother.

TABLE I
Composition of diets*
(Grams per day)

Diet Articles	Case 1 S C C								Case 2 L C Y				Case 3 L I L
	1	2	3	4	5	6	7	8	1	2	3	4	1
Flour	150	325	225	170	100	100	100	100	200	300	300	200	150
Rice	30	60	60	45	150	150	150	150	100	200	100	100	40
Millet	75	75	75	56					100			50	50
Bean curd					50	50	50	50					
Kan Fen					20	20	20	20					
Peanut					20	20	20	20					
Pork	100	100	100	75	80	80	80	80	120	30	30	120	60
Beef													30
Egg, hen's						30		90		200	200		
Cabbage	100	100	100	75					100	100	100	100	50
Spinach	50	50	50	38	50	50	50	50	100		200	100	
Kai Tsai										200	200		
Turnip	50	50	50	38					50			50	40
Carrot													50
Sweet potato	50	240	140	105					100			100	100
White potato									50			50	
Bean sprout					50	50	50	50					
Pickled cucumber					10	10	10	10					
Pear	100	100	100	75									200
Apple	100	100	100	75	100	100	100	100	100	100	100	100	100
Persimmon	100	100	100	75									
Sesame oil	30	30	30	22	30	30	30	30	40	40	40	40	20
Soybean sauce					20	20	20	20					
Sugar					10	10	10	10					
Sodium chloride	5.8	6	6	4	5	5	5	5	8	8	8	8	4
Total calorie	1656	2605	2134	1600	1733	1777	1733	1866	2343	2604	2245	2163	1490
Carbohydrate	244	448	347	261	248	248	248	248	361	403	324	323	248
Fat	53	56	54	40	58	61	58	67	68	70	70	67	36
Protein (N×6.25)	48.9	70.4	63.2	48.1	55.6	55.3	53.8	62.2	67.2	83.7	74.7	61.4	43.6
Calcium	0.146	0.249	0.230	0.208	0.245	0.176	0.149	0.198	0.232	0.424	0.393	0.245	0.167
Phosphorus	0.632	0.832	0.809	0.636	0.686	0.663	0.594	0.778	0.874	1.046	0.994	0.878	0.549

* Calorie, carbohydrate and fat are calculated values (Outlines of Diets of the Peiping Union Medical College Hospital, Peiping, 1937, 3rd edition) Protein, calcium and phosphorus are analyzed values

RESULTS

Case 1, S C C, osteomalacia

I The antirachitic potency of the previous dietary and the lack of such potency in the experimental diets with resulting vitamin D depletion Data obtained in periods 1 to 12 (Table II and Figure 1) indicate that, when the patient was first under metabolic observation on the experimental vitamin D-deficient diets, a good calcium and phosphorus balance was obtained. The calcium retention in periods 1 to 4 amounted to an average of 54 per cent of the intake and the phosphorus retention in the same periods, 58 per cent. Urinary phosphorus became almost nil in period 4, while considerable amounts of calcium appeared in the urine in periods 3 to 4. The disappearance of urinary phosphorus was probably in part related to the exceptionally high retention of nitro-

gen in those periods which required an equivalent amount of phosphorus for deposition as soft tissues, leaving very little phosphorus for urinary elimination. Likewise, a shortage of phosphorus for deposition with calcium in the bone was created, thus accounting for the increased elimination of urinary calcium. However, the excellent mineral balances indicate that the pathological disturbance of osteomalacia in this case was in the process of reparation, presumably under the influence of vitamin D that was derived from the diet and medication administered in the pre-experimental period. Although there was no exact record as to how much vitamin D was supplied in the previous dietary, it is certain that a number of hens' eggs were given. It was also recorded that she received altogether 40 cc of cod liver oil in four doses on two different dates. It may

TABLE II

Case 1 S. C. C. Calcium and phosphorus metabolism during vitamin D depletion, after the administration of small doses of vitamin D and after supplementing basal diets with eggs

Date 1938-1939	Period four-day	Calcium, average daily				Phosphorus, average daily				Nitrogen bal. average daily	Serum			Remarks
		In take	Urine	Stool	Balance	In- take	Urine	Stool	Balance		Cal- cium	Phos- phorus	Phos- pha- tase	
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.		mgm. per cent	mgm. per cent	units	
November	8-11	1347	1	513	833	582	57	250	275	1.01	8.96	3.89	12.67	Diet 1
	12-15	2 1346	2	583	761	632	46	244	342	1.08	9.06	3.49		Diet 1
	16-19	3 1449	78	663	708	832	37	286	519	3.87	9.06	3.13	10.99	Diet 2
	20-23	4 1449	130	608	711	832	8	296	528	2.71	9.32	2.70		Diet 2
	24-27	5 1449	2	658	789	832	153	292	385	2.01	8.49	2.44		Diet 2
	28-1	6 1449	2	732	715	832	174	299	359	2.46	8.70	4.22	10.08	Diet 2
December	2-5	7 1449	7	802	640	832	258	319	255	2.08	8.92	4.24		Diet 2
	6-9	8 1449	4	905	540	832	272	329	231	2.03	8.07	3.85		Diet 2
	10-13	9 1430	0	981	447	809	366	298	145	1.45	7.93	3.94		Diet 2
	14-17	10 1430	0	1125	305	809	348	344	117	2.17	8.01	3.68		Diet 3
	18-21	11 1430	1	961	468	809	311	282	216	3.05	8.62	3.53		Diet 3
	22-25	12 1430	1	1125	304	809	342	338	129	2.07	8.45	3.41		Diet 3
	26-29	13 1430	2	1182	246	809	342	308	159	0.95	8.08	3.05	9.89	Diet 3 Citrate mixture
January	30-2	14 1408	1	1128	279	636	378	279	-21	0.75	8.40	3.03		Diet 4 Citrate mixture
	3-6	15 1408	4	1221	183	636	375	274	-13	0.68	8.65	2.94		Diet 4 Citrate mixture
	7-10	16 1408	4	1004	400	636	401	245	-10	-0.39	8.65	2.84		Diet 4
	11-14	17 1396	2	1311	83	592	333	308	-49	-0.38	8.28	2.86	8.42	Diet 4
	15-18	18 1408	5	1018	385	636	376	346	14	1.22	8.56	3.72		Diet 4 NH ₄ Cl mixture
	19-22	19 1408	41	1248	119	636	412	308	-84	0.92	7.84	2.32		Diet 4 NH ₄ Cl mixture
	23-26	20 1408	98	1084	226	636	456	222	-42	1.44	7.92	2.42		Diet 4 NH ₄ Cl mixture
	27-30	21 1408	15	1249	144	636	270	264	103	0.90	8.50	2.32		Diet 4
February	31-3	22 1408	8	1372	28	636	329	312	-5	0.55	8.74	2.43		Diet 4
	4-7	23 1245	13	1054	178	686	357	292	37	2.28	8.70			Diet 5
	8-11	24 1245	15	1014	216	686	334	282	70	1.77	8.28	2.19		Diet 5
	12-15	25 1245	2	1111	132	686	401	310	-23	0.93	8.83	2.15		Diet 5
	16-19	26 1245	2	891	352	686	330	280	76	1.35	8.60	2.23		Diet 5
	20-23	27 1220	3	881	334	564	237	238	89	0.50	8.55	2.30		Diet 5 Viganol 1/24 cc. daily
	24-27	28 1245	52	709	484	686	152	231	303	2.70	8.29	2.62		Diet 5 Viganol 1/24 cc. daily
	28-3	29 1245	45	834	366	686	103	453	130	1.50	7.20	2.18		Diet 5 Viganol 1/24 cc. daily
March	4-7	30 1245	6	607	632	686	112	243	331	1.58	7.70	2.90	14.88	Diet 5
	8-11	31 1245	6	534	705	686	94	279	315	1.97	8.70	2.60		Diet 5
	12-15	32 1245	0	454	791	686	116	220	350	0.98	8.56	3.16		Diet 5
	16-19	33 1245	0	630	615	686	131	266	259	0.91	8.05	2.76		Diet 5
	20-23	34 1245	0	876	419	686	142	317	226	1.48	8.10	2.41	14.77	Diet 5
	24-27	35 1245	0	768	477	686	189	248	240	2.17	8.16	2.51		Diet 5
	28-31	36 1245	4	750	491	686	191	242	253	1.70	8.04	2.24		Diet 5
April	1-4	37 1176	4	761	411	663	158	262	243	1.16	8.07	2.64		Diet 6 (1 egg)
	5-8	38 1176	8	922	246	663	224	244	97	1.25	8.55	2.60	11.68	Diet 6 (1 egg)
	9-12	39 1176	4	846	326	663	265	285	113	0.63	8.51	2.25		Diet 6 (1 egg)
	13-16	40 1176	5	692	479	663	261	256	166	0.33	8.36	2.58		Diet 6 (1 egg)
	17-20	41 1149	0	1008	141	594	252	349	-7	0.37	8.78	2.42		Diet 7
	21-24	42 1124	10	1016	98	574	250	313	-39	0.92	8.06	2.06	13.44	Diet 7
	25-28	43 1149	0	882	267	594	252	269	73	1.37	8.50	2.46		Diet 7
	29-3	44 1108	0	954	244	778	248	350	180	0.75	8.31	2.42		Diet 8 (3 eggs)
May	3-6	45 1128	0	698	430	549	262	226	61	-0.62	8.56	2.78		Diet 8 (3 eggs)
	7-10	46 1140	0	882	258	475	251	211	13	-0.40	8.02	2.63		Diet 8 (3 eggs)
	11-14	47 1198	6	693	499	778	178	282	189	3.06	8.02	2.42		Diet 8 (3 eggs)
	15-18	48 1198	42	535	601	778	184	303	191	1.89	8.28	2.63		Diet 8 (3 eggs)
	19-22	49 1149	7	561	581	594	156	246	192	1.04	8.22	2.65		Diet 7
	23-26	50 1149	6	653	490	594	175	270	149	1.01	8.96	2.60		Diet 7
	27-30	51 1149	0	632	517	594	168	262	163	1.18	8.44	2.52		Diet 7
	31										8.24	2.59		

be surmised that before the present study she received some vitamin D which though small in amount, was sufficient to bring about the favorable calcium and phosphorus retention observed at the beginning of the experiment.

In order to see how long this degree of calcium and phosphorus retention would continue and to determine whether the experimental diets would maintain this favorable state of affairs observation was extended for 12 periods, or almost seven weeks, without modification of the regimen. It is plainly seen that the urine calcium disappeared

and the fecal calcium increased progressively so that by the end of period 12 it had become twice as much as that at the beginning and the positive calcium balance was reduced to only 21 per cent of the intake. Simultaneously the phosphorus balance was also reduced as the result of increased excretion both in the urine and in the stool that in the former being more marked. There was a slight reduction of serum calcium and a more marked diminution of serum inorganic phosphorus. The initial drop of serum inorganic phosphorus in periods 1 to

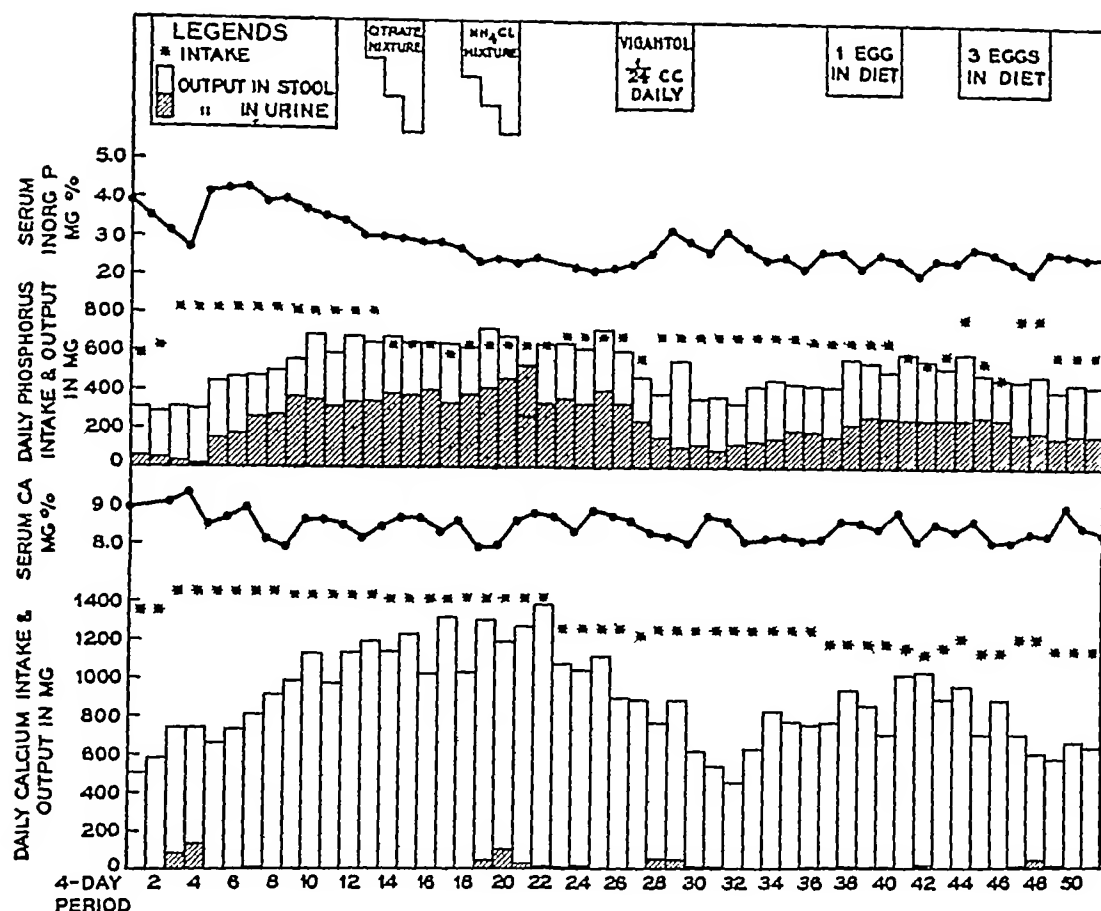


FIG 1 CASE 1, S C C CALCIUM AND PHOSPHORUS METABOLISM DURING VITAMIN D DEPLETION, AFTER THE ADMINISTRATION OF SMALL DOSES OF VITAMIN D AND AFTER SUPPLEMENTING BASAL DIETS WITH EGGS

4 was probably related to the relatively low phosphorus intake and the rapid deposition in the soft tissues and in the bones as the result of high nitrogen and calcium retention, while the subsequent decline was probably due to vitamin D depletion

These data demonstrate clearly that the beneficial effect of the hospital diet on mineral metabolism, together with a few doses of cod liver oil, was real, and it lasted for approximately seven weeks. On the other hand, the experimental vitamin D-deficient diets were unable to maintain the good calcium and phosphorus balance initiated prior to their institution

II The effect of acid and alkali salt mixtures and extension of observations on vitamin D depletion We are not primarily concerned with the effect of acid and alkali in this part of the

study. The data were included in the present communication merely for the sake of continuity. It may be noted, however, that daily administration of 200 to 400 cc of a solution containing 0.6 N of sodium citrate and 0.9 N of citric acid during periods 13 to 15 and 60 to 180 milliequivalents of a mixture of ammonium chloride and ammonium carbonate during periods 18 to 20 did not affect the calcium and phosphorus metabolism in any important way. The urine, however, contained considerable amounts of calcium and an augmented quantity of phosphorus during the period of ammonium chloride mixture administration. These were expected from the acid effect. The calcium retention showed a further tendency to diminish, irrespective of the acid and alkali administration, so that by the end of period 22 the calcium retention became almost nil. The

phosphorus balance was slightly negative during these periods because of the somewhat reduced intake, together with the poor calcium and nitrogen balance. The negative phosphorus balance was slightly exaggerated by the ammonium chloride mixture administration in periods 19 to 20. The serum calcium fluctuated around 8.5 mgm. per cent but the inorganic phosphorus declined further to below 2.5 mgm. per cent, partly from reduction of phosphorus intake in the diet and partly from continued vitamin D depletion. We are therefore, inclined to consider that, besides some minor changes the data obtained during periods 13 to 22 represent an extension of the observation made in the first 12 periods. In other words the calcium retention in the course of 22 periods on the experimental diets changed from 54 per cent of the intake to only 2 per cent. This is undoubtedly the result of vitamin D depletion.

III The effect of a minimal dose of Vigantol
Vigantol was diluted with olive oil 1 to 24 and 1 cc. of this diluted solution containing 500 international units of vitamin D was given daily during periods 26 to 29. The effect on the calcium and phosphorus metabolism was promptly shown up. The fecal calcium decreased rapidly and urinary calcium began to appear. The calcium retention which averaged not more than 15 per cent of the intake in periods 23 to 25 just prior to Vigantol administration increased progressively reaching its maximum in period 32, twelve days after Vigantol was discontinued. The calcium retention then represented almost 64 per cent of the intake. The phosphorus retention ran a parallel course, increasing from only 4 per cent of intake in periods 23 to 25 to 51 per cent in period 32. This was mainly the result of reduction of urine phosphorus. The serum calcium did not show any important change but the serum inorganic phosphorus was distinctly improved.

However the beneficial effect of such a small dose of vitamin D for a limited period was obviously not a lasting one, as shown by the observations during periods 33 to 36. The calcium retention definitely diminished and the same was true with the phosphorus balance. The serum inorganic phosphorus also declined. Time did not permit extension of the observation to the

time when the calcium and phosphorus retention would resume the original low value.

IV The effect of one and three hens' eggs
When one egg was added to the diet during periods 37 to 40 no definite effect could be discerned in the calcium and phosphorus metabolism. It is estimated that one egg yolk at its best contained no more than 60 international units of vitamin D, an amount which would be hardly detectable in human experiment. However immediately after the single egg was withdrawn, the calcium retention was distinctly poorer in periods 41 to 43 and the fecal calcium reached the same original high level as that before Vigantol was administered. This may be taken to mean that the antirachitic potency of a single egg did help in checking the declining tendency of the calcium balance although it was not sufficient to reverse the process.

When three eggs were included in the diet during periods 44 to 48 unequivocal changes in the calcium and phosphorus metabolism were observed. The fecal calcium decreased with an increase of calcium retention from an average of 15 per cent in periods 41 to 43 to over 50 per cent of the intake in periods 48 to 49. Appreciable amounts of calcium appeared in the urine in the last period of the egg ingestion. Toothache and excision of a radicular cyst disturbed the food intake, resulting in negative nitrogen balance and very poor phosphorus retention during period 45 to 46. However, subsequent observation showed definite improvement in phosphorus retention due chiefly to reduction of urinary phosphorus. The serum calcium and inorganic phosphorus showed no significant changes. No attempt was made to determine the exact duration of the beneficial effect of the three-egg regimen.

Case 2 L C Y osteomalacia and rheumatic heart disease

I The exhibition of vitamin D depletion on experimental vitamin D-deficient diet
This patient received 500 cc. of cod liver oil in June and just passed through the summer before the present investigation began in October. It is, therefore not astonishing to see a positive calcium balance even when she was put on an intake of only 232 mgm. per day (periods 1 to 2). When the calcium intake was raised to over 1,200 mgm.,

TABLE III

Case 2 L C Y Calcium and phosphorus metabolism during vitamin D depletion, after supplementing basal diets with eggs and after the administration of vitamin D in minimal and full doses

Date 1938-1939	Period four-day	Calcium average daily				Phosphorus average daily				Nitrogen balance average daily	Serum			Remarks		
		In take	Urine	Stool	Bal ance	In take	Urine	Stool	Bal ance		Cal cium	Phos phorus	Phos pha tase			
		mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm		grams per cent	mgm per cent	units			
October	11-14	1	232	1	146	85	874	411	373	90	0.69	7.68	4.25	13.27	Diet 1	
	15-18	2	232	2	103	127	874	411	281	182	1.21	8.15	4.13		Diet 1	
	19-22	3	1232	2	616	614	874	269	400	205	0.04	8.22	4.36	11.46	Diet 1	
	23-26	4	1232	1	718	513	874	138	509	227	1.06	7.83	4.20		Diet 1	
	27-30	5	1232	3	685	544	874	194	388	292	0.49	8.25	4.09		Diet 1	
	31-3	6	1232	4	811	417	874	272	429	173	0.62	8.32	3.97		Diet 1	Citrate mixture
November	4-7	7	1232	3	940	289	874	282	485	107	1.02	8.21	3.94		Diet 1	Citrate mixture
	8-11	8	1232	6	735	491	874	340	386	148	1.09	8.26	3.88		Diet 1	Citrate mixture
	12-15	9	1232	2	1002	228	874	384	475	15	0.71	8.13	3.70	10.07	Diet 1	
	16-19	10	1232	3	1010	219	874	352	467	55	0.88	8.40	3.85		Diet 1	
	20-23	11	1232	2	1001	229	874	332	484	58	1.12	8.25	3.43		Diet 1	
	24-27	12	1232	5	785	442	874	421	368	85	4.38	8.03	3.66		Diet 1	NH ₄ Cl mixture
December	28-1	13	1232	32	1089	111	874	494	432	-52	1.42	8.35	3.24		Diet 1	NH ₄ Cl mixture
	2-5	14	1232	7	1140	85	874	346	514	14	1.19	8.66	2.81	7.77	Diet 1	
	6-9	15	1232	3	908	321	874	399	397	78	1.50	7.87	3.39		Diet 1	
	10-13	16	1232	0	1164	68	874	424	520	-70	1.13	8.08	3.50		Diet 1	
	14-17	17	1242	3	1410	11	1046	428	546	72	3.19	7.76	3.41	6.54	Diet 2 (6 eggs)	
	18-21	18	1393	1	1084	308	994	494	358	142	1.51	8.46	3.31		Diet 3 (6 eggs)	
	22-25	19	1393	3	1004	386	994	438	349	107	1.73	8.44	3.44		Diet 3 (6 eggs)	
	26-29	20	1393	12	941	440	994	379	380	235	1.59	8.44	3.50	5.12	Diet 3 (6 eggs)	
	30-2	21	1393	31	624	738	994	300	324	370	1.63	8.30	3.46		Diet 3 (6 eggs)	
January	3-6	22	1393	53	495	845	994	303	308	383	1.21	8.17	3.74		Diet 3 (6 eggs)	
	7-10	23	1393	116	470	807	994	269	286	439	1.68	8.61	3.84		Diet 3 (6 eggs)	
	11-14	24	1393	154	449	790	994	226	299	469	1.27	8.44	4.23	4.81	Diet 3 (6 eggs)	
	15-18	25	1393	158	428	807	994	274	290	430	1.89	8.60	4.48		Diet 3 (6 eggs)	
	19-22	26	1393	188	481	724	994	260	321	413	1.81	8.42	4.51		Diet 3 (6 eggs)	
	23-26	27	1393	108	422	863	1338	517	260	361	2.40	8.27	4.66		Diet 3 (6 eggs) + Na ₂ PO ₄	
	27-30	28	1393	97	447	849	1338	532	300	306	2.02	8.48	4.97	2.55	Diet 3 (6 eggs) + Na ₂ PO ₄	
	31-3	29	1393	94	442	857	1338	555	324	659	1.95	8.67	4.74		Diet 3 (6 eggs) + Na ₂ PO ₄	
February	4-7	30	1378	106	410	862	1278	546	279	453	1.38	8.48	—		Diet 3 (6 eggs) + Na ₂ PO ₄	
	8-11	31	1239	142	502	595	860	334	258	268	-0.55	8.61	4.59		Diet 4	
	12-15	32	1245	43	508	694	878	354	303	221	0.63	8.93	4.28		Diet 4	
	16-19	33	1245	21	491	473	878	256	278	344	1.58	8.92	4.01		Diet 4	
	20-23	34	1245	23	663	559	878	327	303	248	-0.49	8.15	3.76		Diet 4	
	24-27	35	1245	23	681	541	878	289	318	271	-0.08	8.00	3.97		Diet 4	
March	28-3	36	1245	16	720	509	878	357	343	178	-0.24	8.34	3.57		Diet 4	
	4-7	37	1245	0	763	482	878	354	312	212	0.31	8.52	3.72	6.14	Diet 4	
	8-11	38	1245	5	910	330	878	344	354	180	0.61	8.59	3.72		Diet 4	
	12-15	39	1245	0	896	349	878	412	362	104	-0.91	8.68	3.58		Diet 4	
	16-19	40	1245	0	959	286	878	373	358	147	0.99	8.01	3.41		Diet 4	
	20-23	41	1245	0	1032	213	878	400	364	114	-0.14	8.15	3.36	7.68	Diet 4	
	24-27	42	1245	0	1025	220	878	369	369	140	1.31	8.20	3.18		Diet 4	
	28-31	43	1245	0	1132	113	878	323	396	159	1.46	8.22	2.70		Diet 4	
April	1-4	44	1245	6	1045	194	878	304	366	208	1.61	7.66	3.08		Diet 4	Vigantol 1/24 cc daily
	5-8	45	1245	10	992	243	878	354	344	180	1.57	8.42	3.22		Diet 4	Vigantol 1/24 cc daily
	9-12	46	1245	2	939	304	878	303	344	231	2.59	8.94	3.32	5.96	Diet 4	Vigantol 1/24 cc. daily
	13-16	47	1245	2	848	395	878	299	306	273	1.40	8.61	3.33		Diet 4	Vigantol 1/24 cc daily
	17-20	48	1245	3	710	532	878	260	310	308	-1.40	8.31	3.27		Diet 4	
	21-24	49	1245	3	732	510	878	254	308	316	0.86	8.38	2.99		Diet 4	
	25-28	50	1245	0	742	503	878	287	309	282	1.05	8.74	3.60	5.93	Diet 4	
	29-2	51	1245	0	808	437	878	297	297	284	0.90	8.50	3.12		Diet 4	
May	3-6	52	1245	0	861	384	878	352	316	210	0.05	8.64	3.75		Diet 4	
	7-10	53	1245	0	896	349	878	322	346	210	1.00	8.09	3.65		Diet 4	
	11-14	54	1245	0	734	511	878	326	285	267	1.18	8.55	3.68		Diet 4	
	15-18	55	1245	0	745	500	878	278	325	275	1.42	8.36	3.22		Diet 4	
	19-22	56	1245	0	865	380	878	230	379	269	1.78	8.56	3.48		Diet 4	Vigantol 1 cc daily
	23-26	57	1245	21	644	580	878	200	287	396	1.65	8.83	3.23		Diet 4	Vigantol 1 cc daily
	27-30	58	1245	11	580	654	878	232	259	387	0.95	8.44	3.84		Diet 4	Vigantol 1 cc daily
	31-3	59	1245	27	494	724	878	192	244	442	1.87	8.54	4.03		Diet 4	Vigantol 1 cc daily
June	4-7	60	1245	82	434	729	878	189	236	453	1.79	8.22	4.32	4.05	Diet 4	Vigantol 1 cc. daily

the average calcium retention in periods 3 to 5 amounted to 45 per cent of the intake. The average phosphorus retention of the corresponding periods was almost 28 per cent of the intake. The urinary phosphorus ran low. The serum calcium was around 8 mgm per cent but the inorganic phosphorus was more than 4 mgm per cent.

The effects of 100 to 300 cc of a solution containing 0.6 N sodium citrate and 0.9 N citric acid and a solid mixture of 60 to 120 milliequivalents of ammonium carbonate and of ammonium chloride were studied during periods 6 to 8 and 12 to 13, respectively. As in Case 1, we cannot attribute any important changes to the use of these salt mixtures other than a slight drainage

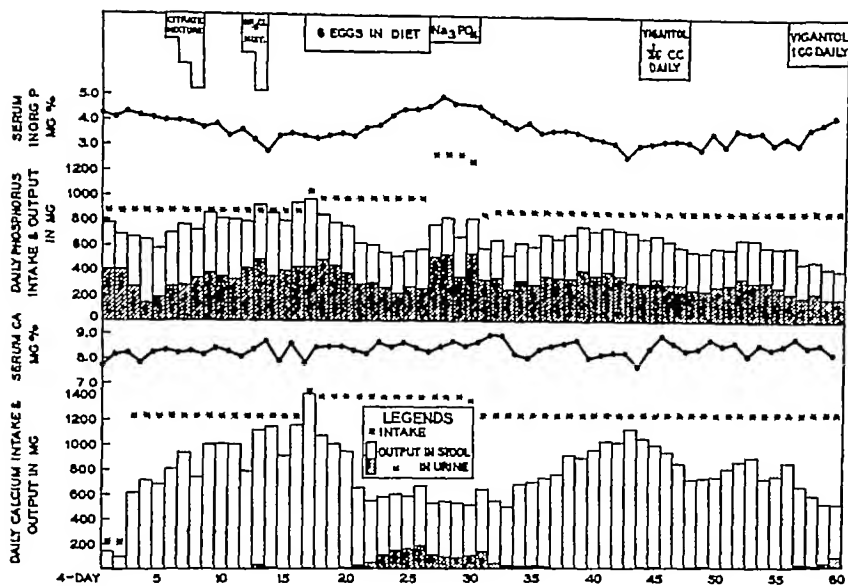


FIG. 2. CASE 2, L. C. Y. CALCIUM AND PHOSPHORUS METABOLISM DURING VITAMIN D DEPLETION AFTER SUPPLEMENTING BASAL DIETS WITH EGGS AND AFTER THE ADMINISTRATION OF VITAMIN D IN MINIMAL AND FULL DOSES

of urinary calcium and a definite increase of urinary phosphorus, together with slight impairment of phosphorus retention during the periods of ammonium chloride administration.

It is, however, clear that throughout periods 3 to 16 there was an uninterrupted trend of reduction in both the calcium and the phosphorus retention. The calcium retention in period 16 measured less than 6 per cent of the intake. This was entirely due to a progressive increase in fecal calcium. Both the urine and the fecal phosphorus increased the urine more so. The phosphorus balance during period 16 was definitely negative. The serum calcium did not change but the inorganic phosphorus dropped to a distinctly lower level. Again these findings may be taken as characteristic effects of vitamin D depletion. The experimental diets were sufficiently devoid of vitamin D to allow such depletion to take place.

II The effect of six eggs Two hundred grams of egg approximately equivalent to six average

sized Chinese hens' eggs contained 80 grams of yolk, which was estimated to contain not more than 400 international units of vitamin D. This was given daily during periods 17 to 26. There was a sudden progressive reduction of fecal calcium and a similar reduction of urinary and fecal phosphorus. With further reduction of fecal calcium urinary calcium began to appear and reached a significant proportion by the end of the last period of egg ingestion. The result of these changes was a marked increase of calcium and phosphorus retention which reached their maximum—namely about 61 per cent of the intake—in the sixth period of egg ingestion. This high degree of calcium retention was maintained for the following 4 periods. The changes in the serum calcium were insignificant but the inorganic phosphorus rose to a perfectly normal level.

When more phosphorus was given in the form of a solution of trisodium phosphate in periods 27 to 30 the urinary calcium decreased slightly

and there resulted a slight further increase of calcium retention. The phosphorus retention was also further increased but most of the excess phosphorus appeared in the urine.

The optimal calcium balance continued unchanged for about 7 periods after the egg ingestion was discontinued. Then the effect of the egg diet began to wear off. First of all the urine calcium decreased and later disappeared. The fecal calcium increased steadily. Both the urine and the fecal phosphorus increased. The serum calcium decreased slightly and the serum inorganic phosphorus once more came down to a very low level. By period 13 the effect of the egg diet may be considered to have entirely disappeared. The calcium retention then was only 9 per cent of the intake compared with the average optimal retention of 57 per cent during periods 22 to 26.

To summarize this experiment six eggs daily for forty days brought about a maximal calcium and phosphorus retention in twenty-four days after the commencement of their ingestion. The maximal retention was maintained for forty-eight days or twenty-eight days after the egg ingestion was stopped. It took sixty-eight days after its discontinuation for the beneficial effect to disappear completely.

III The effect of minimal and full doses of

Vigantol. One cc of a diluted *Vigantol* (1 24) containing 500 international units of vitamin D was given during periods 44 to 47. Its action on the calcium and phosphorus balances was seen immediately and the maximal effect was obtained in the period immediately after the drug was discontinued. This improved calcium and phosphorus retention continued for 3 more periods before it began to decline. A slight increase of the serum calcium and inorganic phosphorus was also noticeable. The definite but transient effect of the small amount of *Vigantol* observed in the present case resembled that observed in Case 1 in a remarkably close manner.

When the full dose of *Vigantol*, 1 cc containing 12,000 international units of vitamin D, was given in the last 5 periods, the calcium retention was naturally further improved and calcium began to appear in the urine. The urine and fecal phosphorus decreased, resulting in more favorable phosphorus balance. The serum inorganic phosphorus was raised to over 4 mgm per cent. There was no time to observe the maximal degree of improvement and the course of depletion.

Case 3, L T L, osteomalacia and rickets

The vitamin D-deficient and calcium-low diet and the production of negative mineral balances as a sign of vitamin D depletion. This patient

TABLE IV

Case 3 L T L Calcium and phosphorus metabolism during vitamin D depletion while on a low calcium diet

Date 1937-1938	Pe- riod four day	Calcium average daily				Phosphorus, average daily				Ni- tro- gen bal- ance average daily	Serum			Remarks
		In take	Urine	Stool	Bal ance	In- take	Urine	Stool	Bal ance		Cal- cium	Phos- phorus	Phos- pha- tase	
		mgm	mgm	mgm	mgm	mgm	mgm	mgm	mgm	grams	mgm per cent	mgm per cent	units	
November	8-11	1	167	8	46	113	549	234	156	0.91	6.56	3.69	11.00	Diet 1
	12-15	2	167	8	42	117	549	274	143	0.92	6.94	4.09		Diet 1
	16-19	3	167	6	62	99	549	308	193	0.47	5.66	4.29		Diet 1
	20-23	4	161	65	78	18	538	331	204	3	0.08	6.64	4.01	Diet 1 NH ₄ Cl
	24-27	5	167	86	110	-29	549	412	226	-89	-0.30	6.12	4.15	Diet 1 NH ₄ Cl
	28-1	6	167	89	123	-45	549	452	227	-130	-0.06	6.07	3.99	Diet 1 NH ₄ Cl
December	2-5	7	167	84	137	-54	549	400	242	-93	0.31	5.92	4.01	Diet 1 NH ₄ Cl
	6-9	8	167	32	139	-4	549	379	274	-104	0.98	6.21	4.07	Diet 1
	10-13	9	167	19	136	12	549	386	195	-32	1.09	5.92	4.15	Diet 1
	14-17	10	167	4	172	-9	549	374	250	-75	0.97	5.74	4.09	Diet 1 NaHCO ₃
	18-21	11	167	6	208	-47	549	373	290	-114	0.63	5.96	4.24	Diet 1 NaHCO ₃
	22-25	12	167	7	198	-38	549	371	226	-48	0.65	5.66	4.06	Diet 1 NaHCO ₃
	26-29	13	167	13	140	14	549	330	233	-14	0.86	5.42	3.96	Diet 1 NaHCO ₃
	30-2	14	167	19	208	-60	549	326	290	-67	0.96	5.76	3.87	Diet 1
January	3-6	15	167	17	227	-77	549	417	261	-129	0.73	—	4.01	Diet 1
	7-										5.70	4.06	10.44	

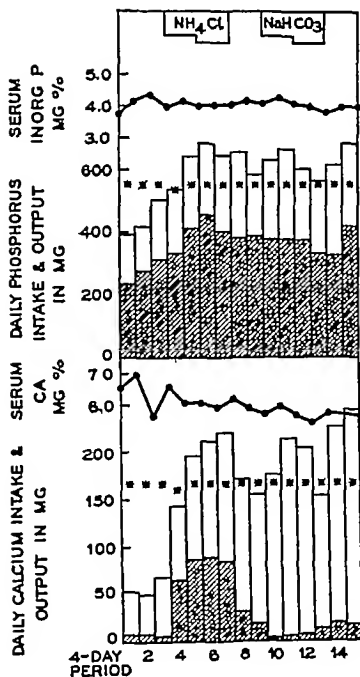


FIG. 3 CASE 3, L. T. L. CALCIUM AND PHOSPHORUS METABOLISM DURING VITAMIN D DEPLETION WHILE ON A LOW CALCIUM DIET

Legends as in other figures.

had been on a full hospital diet for one month in the orthopedic service prior to the present study. The diet included eggs, milk and sometimes liver. No vitamin D medication or ultraviolet irradiation was prescribed. When she was put on the low calcium and vitamin D-deficient diet, her calcium and phosphorus metabolism was found to be remarkably conservative. She was able to retain on the average 110 mgm. of calcium out of a daily intake of only 167 mgm. in periods 1 to 3. The phosphorus retention was equally favorable. The serum calcium rather low to start with, showed a tendency to decline further with the low calcium intake. The serum inorganic phosphorus however, was maintained in the neighborhood of 4 mgm per cent.

Ammonium chloride and sodium bicarbonate in doses of 30 to 40 milliequivalents daily were given during periods 4 to 7 and 10 to 13 respectively. The marked increase of urinary calcium and phosphorus was attributed to the action of the acid producing salt, ammonium chloride, but no change could be assigned to sodium bicarbonate. This opinion was well corroborated by the findings of Farquharson and associates (6).

When one peruses the figures for fecal calcium, ignoring the urinary calcium for the time being, a staircase rise of fecal calcium throughout periods 4 to 15 becomes apparent. This progressive increase of fecal calcium was in no way related to the acid and alkali administration. The calcium balances during periods 10 to 15, with the exception of period 13, became negative mainly as the result of large stool calcium excretion, which exceeded the intake. The phosphorus balances were also negative, with the urine and stool sharing the drainage. The serum calcium dropped to below 5.5 mgm per cent. All these changes are best explained on the basis of vitamin D depletion. The distinguishing features in this experiment are the low calcium intake and the negative calcium and phosphorus balances. These are the circumstances which must have existed in all cases of osteomalacia at one time or another, although they are rarely observed in metabolic experiments. The negative calcium and phosphorus balances observed in this patient on a vitamin D-deficient and calcium low diet, although small in extent, are significant, particularly when they are continued. They give us an insight into the pathogenesis of osteomalacia.

Case 4 Baby W H S normal infant

Incipient changes in calcium metabolism following vitamin D depletion. This was a normal infant whose mother was suffering from osteomalacia and who had received 20 cc. of Vigantol in four days about one month before delivery and 30 cc cod liver oil daily for six days immediately after parturition. The calcium and phosphorus metabolism of the mother was undoubtedly benefited by the vitamin D intake, and the baby was protected from fetal rickets. The experiment was primarily designed to demonstrate the maternal transmission of vitamin D in the milk, the details

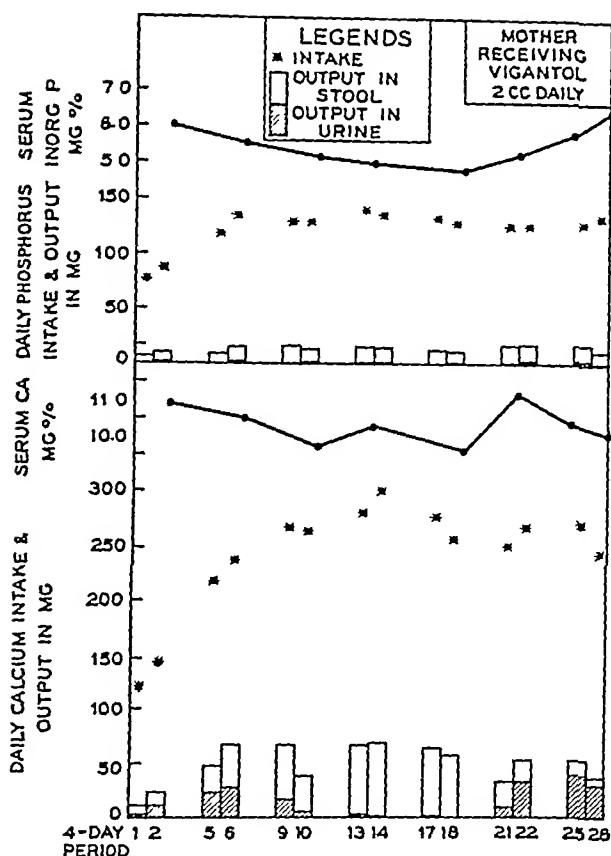


FIG. 4 CASE 4, W H S CALCIUM AND PHOSPHORUS METABOLISM OF A BREAST-FED INFANT SHOWING VITAMIN D DEPLETION WITH SUBSEQUENT REPLENISHMENT, ALL VIA MOTHER'S MILK

of which are reported in a preceding communication (15)¹ We are interested here particularly in the changes of calcium and phosphorus metabolism during the period of vitamin D depletion. The baby received mother's milk exclusively throughout the experiment and the mother was not given any vitamin D until 18 periods of control observation were completed in seventy-two days. A series of events happened during these periods (Figure 4). The urine calcium, which was relatively large in amount, disappeared slowly. The fecal calcium increased but a good calcium retention was still maintained. There was a slight lowering of serum calcium and inorganic phosphorus. However, no significant changes were observed in the phosphorus balances which remained conservative.

¹Part of Figure 4 of the preceding paper (15) is reproduced here for convenience.

When 2 cc of Vigantol were given to the mother daily immediately after period 18 and continued till the end of the experiment, an undetermined amount of vitamin D must have reached the baby through the milk. The reverse phenomenon took place. The fecal calcium decreased almost to nil. Urinary calcium made its appearance again. The net percentage of calcium retention was slightly better. The serum calcium and inorganic phosphorus showed definite increase. The phosphorus balance remained as good as before.

The changes in the calcium and phosphorus metabolism observed in the first 18 periods (actually 10 periods), may be representative of vitamin D depletion. Inasmuch as the mineral retention remained favorable, the shifting of calcium elimination from the urine to the stool might be regarded as an incipient change in the mineral metabolism in the direction of development of rickets or osteomalacia. This was corroborated by the suggestive x-ray findings shortly before vitamin D was given to the mother.

DISCUSSION

The results of the present investigation confirm completely our contention repeatedly expressed in former communications (2, 3, 4, 15) that the diet during the pre-experimental period and the vitamin D medication, even though in small doses and given some time previously, play an important rôle in determining the status of calcium and phosphorus metabolism at the beginning of the experiment. It cannot be doubted that small doses of vitamin D in the form of cod liver oil given for a limited period and the even smaller amount of vitamin D included in the hospital diet for many weeks can bring about a favorable calcium and phosphorus balance. Although this beneficial effect is of limited duration it cannot be appreciated until one is prepared to extend control observation on a vitamin D-deficient diet for a prolonged period. This circumstance has not escaped the attention of early investigators. Hess, Weinstock and Tolstoi (10) realized the influence of the diet during the pre-experimental period on the susceptibility of rats to rickets. The refractory state occasionally encountered in certain groups of rats was found to be due to the

liberal diet during the pre-experimental period. It was overcome and the young rats were rendered susceptible to rickets by feeding the mother and young a less adequate diet throughout the suckling period.

Thus in any investigation on calcium and phosphorus metabolism, the status of the vitamin D factor, past and present, must be clearly defined and segregated lest false interpretations be made. This is not an easy matter, in view of the fact that minimal doses of vitamin D are effective and their effect is sustained long after their discontinuation. Therefore, in order to ascertain and separate the effect of previous vitamin D administration and of subsequent withdrawal control metabolic observations for many periods are essential.

On the basis of the present study we feel justified in concluding that our generally used experimental diets in this series of investigations are deficient in vitamin D. They certainly cannot be relied upon to exert any curative effect on osteomalacia or rickets nor can they maintain whatever beneficial effect may have accrued from a previous regimen. It must be noted however, that these diets essentially cereal vegetable meat-oil conform to the better one of the two types of dietary used by the mass of population in Peiping (8). Common vitamin D-containing foods such as eggs, milk, butter, fish, chicken and liver, etc., are not available to the majority of the population. It is our impression that they derive their full or partial vitamin D requirement from non-dietary source or ultraviolet irradiation from the sun. It is also our opinion that subclinical vitamin D deficiency must be rather prevalent among the population, particularly those who lead a confined life.

The most interesting and instructive feature in the present investigation is related to the metabolic changes following the withdrawal of vitamin D. In a way these changes may be taken to represent the metabolic process in progress in the course of development of rickets or osteomalacia. In the case of calcium these consist of (1) a diminution and, later disappearance of urine calcium (2) a progressive increase of stool calcium as the result of increasing difficulty in the intestinal absorption of the mineral and (3) a progressive decrease of calcium retention on adequate intake

or actual loss of the element on low intake. Simultaneously or somewhat later the phosphorus excretion is increased both in the urine and in the stool. The increased urine phosphorus presumably represents that portion of the absorbed phosphorus which fails to be deposited in the bones because of lack of absorbed calcium. The fecal phosphorus is increased simply by reason of the increased unabsorbed calcium in the bowels. Likewise, phosphorus balance suffers. Both serum calcium and phosphorus decrease but one may be more seriously or sooner affected than the other.

In spite of the fact that the derangements of calcium and phosphorus metabolism in advanced vitamin D deficiency are well known and that the metabolic response to vitamin D therapy in such cases is familiar to the students of rickets and osteomalacia the literature is devoid of any information on the incipient changes that occur in the pre- or subclinical stage of the disease. Skaar's (18) investigation on experimental rickets in dogs was perhaps the only published work which included metabolic observations in the course of development of rickets. Even in this work the author failed to realize the importance of publishing data on the changes in the paths of excretion of calcium and phosphorus which accompanied the poor retention of the minerals.

The experience gained from the study of the effect of small doses of vitamin D on the calcium and phosphorus metabolism in osteomalacia opens up the possibility of assaying in human beings the relative antirachitic potency of different vitamin D-containing foods. It would be of still greater interest to compare the antirachitic potency of vitamin Ds of different origin a question which has not been answered satisfactorily by clinical experiences with rickets (1, 5, 13, 14).

Heymann (11, 12) demonstrated the presence of vitamin D in the liver and the blood plasma of rabbits for as long as twelve weeks after a single dose of viosterol equivalent to 200 000 international units. This was more or less confirmed by Guerrant and associates (7) in growing calves and by Vollmer (19) in human beings. These facts about the storage of vitamin D following oral administration provide a logical explanation for the prolonged duration of the antirachitic effect of vitamin D. More work must be done however to determine the extent of

in relation to the size of the dose administered. Further work along the lines started in the present investigation would also answer the question as to whether the duration of the antirachitic effect of a given dose of vitamin D would be proportional to the size of the dose.

SUMMARY

Calcium and phosphorus metabolism was studied in 3 patients with osteomalacia and 1 normal infant born of an osteomalacic mother. Prolonged observation was made while the patients were on vitamin D-deficient diets. The infant was given breast milk exclusively, while the mother was on a similar vitamin D-deficient diet. At the beginning all 4 cases showed conservative calcium and phosphorus balances, due presumably to prior vitamin D store either from the hospital diet or from medication as cod liver oil. But in the course of time the following metabolic changes were observed. The urine calcium decreased and then disappeared, the fecal calcium became progressively increased, and the calcium balance was reduced or became negative when the calcium intake was low. The phosphorus metabolism followed a similar unfavorable course, both the urine and the fecal phosphorus were increased. The serum calcium and inorganic phosphorus usually diminished but slightly in the course of these observations.

These metabolic changes were interpreted as evidence of vitamin D depletion. When they were put on the experimental diets, which were sufficiently devoid of vitamin D to allow the depletion of the prior store, whatever beneficial effect they derived from the previous regimen disappeared in the course of time, giving place to all the metabolic changes enumerated above. These biochemical alterations are regarded as early signs of vitamin D depletion, as they must go on for some time before clinical evidence of such deficiency can be elicited. They are helpful in providing material for reconstructing the metabolic processes that underlie the development of rickets and osteomalacia.

Experiments with very small doses of vitamin D in the form of Vigantol or the addition of eggs to the basal diet, as in Cases 1 and 2, demonstrated the efficacy of such minimal doses of vita-

min D, and confirmed the supposition that the favorable metabolic behavior observed at the beginning of the experiments in all the cases was due to previous vitamin D store which was given up very slowly.

CASE ABSTRACTS

Case 1, S C C, Hospital Number 64728, a Chinese married woman of 18, was admitted to the obstetrical service on October 13, 1938, after she had been in labor for thirty hours. She gave a history of pain in the thighs and difficulty in walking for about three and a half years. She had been married at the age of 14. Her menstruation started shortly after marriage and she became pregnant for the first time just after the first period. She was apparently well in the first trimester of the pregnancy but she began to have pain in the thighs and difficulty in walking during the remaining course of pregnancy. She gave birth to the first child without dystocia in October 1935. Her symptoms subsided for three months after parturition but recurred afterwards. The child was fed on the breasts. Pain in the thighs and difficulty in walking continued till April 1936 when her child died of diarrhea. The same symptoms were present from January to April 1937. The present pregnancy, her second one, commenced in January 1938, together with recurrence of pain in the thighs. The pain was noticeable on walking, which was difficult. She was confined in bed most of the time. There were no symptoms of tetany. Labor pains started approximately thirty hours before admission but no progress was made after repeated examinations by ignorant midwives. She was finally referred to this hospital for prolonged labor. She had productive cough for twenty days in 1937 and again for two weeks last spring. She never had any fever or hemoptysis. The rest of the past history was unimportant. Her diet was always poor, consisting of millet, mixed flour and salted turnip. It was roughly estimated that she got not more than 2,000 calories a day. During the present pregnancy her food intake was even further reduced.

Physical examination on admission revealed that the patient was very small in stature and undernourished. Her weight was 34.1 kgm. She preferred to lie on her side with the lower extremities drawn up and adducted. She complained of pain when her legs were extended and abducted. Gradually, full extension could be accomplished. The adductor muscles of the thighs were spastic and tender. Thoracic cage was asymmetrical with some tenderness over the ribs. There was slight degree of lordosis of the spine, intrusion of both acetabulae and slight knock-knee. Her head organs were normal except for pyorrhea alveolaris. The lungs were clear and the heart of normal size but rapid, with a basal systolic murmur. Blood pressure was 104/70. The abdomen was distended by gravid uterus which was in constant contraction. The fetal heart was irregular. Pelvic measurements were I S 21 cm., I C 23.5 cm., E C 17.5 cm., and T O 8 cm. Rectal and vaginal examinations showed

that the cervical os was fully dilated, the membrane was intact and the fetal head was floating. There was slight pitting edema of the legs. The tendon reflexes were normal and Chvostek's and Trousseau's signs were negative.

Laboratory findings on admission showed that her urine contained some albumin, many white blood cells and occasional red blood cells. Phenolsulphonephthalein excretion was 65 per cent in three hours. Blood count Hemoglobin 11.9 grams red blood cells 4,040,000 and white blood cells 15,950 with 85 per cent polymorphonuclear leukocytes. Stool was positive for ova of *Ascaris*. Blood Wassermann test was negative. Serum calcium was 8.00 mgm. per cent, inorganic phosphorus 1.90 mgm. per cent and phosphatase 10.50 Bodansky units.

After admission the amniotic membrane ruptured and the umbilical cord prolapsed. The fetal heart stopped before the cord could be reduced. The dead fetus was finally delivered by craniotomy. The patient ran a low grade fever in the first week after parturition. Then the temperature went up higher reaching over 39° C. in the next two weeks. During this febrile period the patient did not have much complaint besides slight cough. There was slight dullness in the left upper chest anteriorly and dullness and many crepitant rales in the right lower interscapular area. Blood and urine cultures were sterile. Sputum contained neither pneumococci nor tubercle bacilli. X ray of chest revealed shadows suggestive of advanced pulmonary tuberculosis of right lung and moderate involvement of left side. X ray of the bones at the same time showed slight to moderate degree of osteoporosis of all the bones, triradiate deformity and contraction of the pelvis, old pathological fractures in the pubic and ischial bones, right radius and ulna and a few of the ribs. The urine then contained but a faint trace of albumin. There was a leukocytosis of 11,000 to 25,000. No parasite was ever found in the blood smear. With supportive treatment the fever disappeared in one month. The general condition gradually improved and the physical and x ray findings in the chest also improved. The urine became clear and the leukocytosis disappeared. Through the courtesy of Dr K. T. Lim of the obstetric service, the patient was transferred to the metabolism ward for study on November 7, 1938.

During the postpartum period the patient was given a soft diet and later a high caloric soft diet. These diets including animal protein and eggs, were supposed to be adequate. She received two doses of cod liver oil 10 cc. each, on October 17, 1938, and another two similar doses on November 4, 1938. Throughout the period of study in the metabolism ward the patient did well. However her appetite was limited so that the quantitative diets were of small caloric value. In periods 45 to 46 she developed a swelling of the left cheek and toothache. This was diagnosed as a radicular cyst with infection. Excision was done. This disturbed the metabolic study for 2 periods because the food intake was irregular. The pain in the thighs disappeared gradually after hospitalization. The x ray findings in the lungs also

disappeared. She was discharged on June 1, 1939 in good condition.

Case 2 L. C. Y. Hospital Number 64677 a 45-year old Chinese widow was admitted to this hospital on October 6, 1938, with the complaint of multiple joint pain and muscular aching for more than two years. The patient began to have aching in the lower back and thighs in February 1933. These symptoms continued till September and then subsided. In March 1936 she developed pain in the left lower chest, cough and fever. While the latter symptoms continued pain in the back and the thighs returned in October 1936. The pain gradually involved the whole lower extremities so that finally she could not walk. These symptoms became worse through the winter of 1937 when she had, in addition, numbness of the hands and feet and, on several occasions, spasms of the hands. She came to the outpatient clinic for treatment in June 1938. Rheumatic heart disease, with mitral stenosis but without heart failure, and osteomalacia were diagnosed. Thirty cc. of cod liver oil daily were prescribed and 500 cc. of the oil were supplied. At the same time edema of lower extremities was noticed for twenty days. However the pain in the back and lower extremities was much improved following the medication. She was then able to walk with a stick.

She had had occasional attacks of palpitation of the heart and shortness of breath ever since she was 20 years of age. She gave no definite history of joint pains before the onset of the present illness. She had had attacks of epistaxis since the age of 15 or 16. She had gradually lost her sense of smell in the year preceding her admission. Her menstruation had always been irregular and scanty and it had stopped two years previously. She had been married at 18 but had never become pregnant. Her husband had died ten years previously of chronic cough and hemoptysis of many years standing. Her diet had been poor consisting of millet, white flour and corn flour with salted and fresh vegetables. She had never had any animal food nor any fat or oil for cooking.

Physical examination revealed that the patient was short and undernourished. Weight was 36.8 kgm. and height was 148 cm. She appeared comfortable. Her breath was foul. Post pharyngeal mucosa and nasal mucosa were dry and atrophic. Many teeth were missing a few were carious, and some were loose. There was pyorrhea alveolaris. The right lobe of the thyroid gland was slightly enlarged. The lungs were clear. The heart was slightly enlarged. A rumbling diastolic murmur was present at the apex. P-2 was not accentuated. Blood pressure was 96/60. The abdomen was soft and no viscera were palpable. There was slight edema in the legs. No skeletal deformity or any bone tenderness was observed. No spasm or tenderness was present in the adductor muscles of the thighs. Chvostek's sign was positive but Trousseau's sign was negative. Tendon reflexes were normal. Pelvic measurements were normal.

Laboratory findings Urine was clear and PSP excretion was 70 per cent in two hours. Blood count Hemoglobin 119 grams, red blood cells 4,220,000, white blood cells 7,000. Blood smear was normal. Stool was normal. Blood Wassermann was negative. Serum calcium was 7.68 mgm per cent, inorganic phosphorus 4.25 mgm. per cent and phosphatase 13.27 units. Plasma albumin was 3.47 per cent and globulin 3.45 per cent. Basal metabolic rate was +8 per cent. Electrocardiogram revealed normal mechanism. X-ray examination showed that the frontal area of the heart was 16 per cent oversized, with enlargement in the left auricular region. There was no gross deformity of the bones but moderate degree of osteoporosis and coarsening of trabeculae of all the bones were present. Old pathological fractures were seen in the right 5th and 9th ribs, and the left 6th, 7th and 10th ribs. Old fractures were also present in both scapulae and in the left 2nd metacarpal bone. There was mild biconcavity of the lower thoracic and upper lumbar vertebral bodies and slight deviation of the symphysis pubis towards the right side.

In the course of study, which lasted from October 1938 to June 1939, the patient was much improved. She gained 13 kgm of weight. Her cardiac condition was well compensated. Her atrophic rhinitis was also treated by means of estrone spray with improvement. She was discharged on June 10, 1939 in good condition.

Case 3, L. T. L., Hospital Number 60504, a Chinese girl of 16, was admitted to the orthopedic service on September 28, 1937, with the complaint of pain in the knees for three years and progressive deformity of the lower extremities, spine and chest for one and a half years. The patient was apparently well up to about three years before admission when she began to experience a dull pain in both knee joints, particularly on motion. Symptoms continued but were not severe. In January 1936 the patient had scarlet fever which cleared up in seven days. In March 1936 she began to notice difficulty in extending her knees. One month later her hips also could not be fully extended. Walking became difficult and she was completely confined in bed. Deformity of the lower extremities became progressively worse so that on admission the lower extremities were permanently held at 90° at the hips and at the knees. With the onset of deformity of the lower extremities she had dull pain in the upper spine and in the sternum. This was followed by a bulging deformity in her right upper back and protrusion of the sternum. She denied having had any symptoms of tetany. The patient had been born in a poor family. Hunger had been a common experience. She had had a fair amount of sunlight exposure before she had become incapacitated by her deformities but practically none during the past year and half. Menstruation had not yet started.

Examination showed marked underdevelopment and undernutrition. Weight was 19.6 kgm., height 115.8 cm. She was mentally alert but very quiet. No deformity of skull was present. Both wrists were distinctly enlarged. The thorax was markedly deformed with crumbling of the sternum, so that the distance between the sternal

notch and the xiphoid process measured only 12 cm. There were enlarged costochondral junctions over the lower ribs, obtuse subcostal angle and suggestive Harrison's groove. Marked thoraco-lumbar right kyphoscoliosis was present. The lower extremities showed marked muscular atrophy and contractures, extension at hips and knees was only possible to 90°. Pelvis was markedly contracted with very narrow pubic arch and the transverse outlet measured less than 3 cm. Head organs were essentially normal. Skin was dry and hyperkeratotic. Dark adaptation was only 1/20 of normal. Superficial lymph nodes were palpable. Thyroid gland was diffusely enlarged but more so on the right side. There were no signs of increased vascularity. Lungs showed dullness, with diminished breath sounds in the right upper area. The heart was normal. The abdomen protruded and the liver edge was palpable. Chvostek's sign was positive, but Trousseau's sign was negative and Erb's sign was doubtful.

Routine laboratory examination revealed normal urine, moderate anemia with slight eosinophilia and presence of ova of *Taenia* in the stool. Blood Wassermann test was negative. Serum calcium was 7.5 mgm per cent, inorganic phosphorus 3.1 mgm per cent and phosphatase 7.0 Bodansky units. Plasma albumin was 3.24 per cent and globulin 3.04 per cent. PSP excretion and urea clearance were normal. Basal metabolic rate was +17.3 per cent. X-ray examination showed that all the bones were moderately to markedly osteoporotic. Marked scoliosis of the mid-thoracic spine and markedly exaggerated sacral curve were present. The vertebral bodies were moderately flattened with biconcave deformity. The pelvis was deformed with intrusion of both acetabulae. Costochondral ends of the ribs were broadened. The epiphyseal ends of radius and ulna were moderately expanded and hazy with faint areas of radiolucency. Irregularities and slight cupping were also present. Similar but less marked changes were observed in the epiphyseal ends of the tibia, fibula and femur. There was slight thickening of periosteum of most of the long bones. Her lungs appeared fairly clear.

The patient was first treated on the orthopedic service with Russell's traction to both lower extremities from September 28 to October 31, 1937. She was on full hospital diet, vitamin D medication was purposely withheld. With the kind permission of Drs C. M. Meng and H. C. Fang, the patient was transferred to the metabolic ward for study on November 1, 1937, when her deformities of the lower extremities were considerably improved as the result of traction. Her taeniasis was treated successfully with *Aspidi oleoresina* and proved to be due to *Taenia solium*. Three series of metabolic studies were made, the first of which was reported in the present communication. She was treated, after the first series of study, with ultraviolet irradiation and improved greatly. However, she developed a psychosis which did not improve until some time after discharge on May 30, 1938.

Case 4, W. H. S., Hospital Number 66085, a Chinese male baby, was born of an osteomalacic mother on Feb-

ruary 7 1939 by cesarean section. The history was given under Case 4b of the preceding paper (15)

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OBSERVATIONS ON THE PLASMA PROTHROMBIN AND THE EFFECTS OF VITAMIN K IN PATIENTS WITH LIVER OR BILIARY TRACT DISEASE¹

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There is general agreement that the newer methods for the quantitative determination of plasma prothrombin (1, 2, 3, 4) measure a deficiency that frequently occurs in jaundiced individuals. Numerous studies (5, 6, 7, 8, 9) indicate that a reduction in prothrombin is responsible for the hemorrhagic diathesis which some of these patients exhibit. Some investigators (3, 8, 10, 11, 12, 13) conclude that the prothrombin deficiency and the hemorrhagic state associated with it, can be corrected by the administration of preparations containing the fat soluble anti-hemorrhagic vitamin K, with bile salts.

The use of vitamin K in the treatment of the bleeding tendency in obstructive jaundice was first proposed on theoretical grounds by Quick (14). Actual clinical trial of this therapy was begun independently shortly afterwards by Warner Brinkhous and Smith (2). Butt, Snell and Osterberg (15) and Dam and Glavind (16). These reports and subsequent studies have been extremely enthusiastic regarding the value of crude concentrates of vitamin K administered with bile salts in restoring the prothrombin to normal and in controlling hemorrhage in jaundiced individuals. Very few failures have been reported (13). The purpose of the present investigation was to study critically the efficacy of this new therapy in a large series of consecutive cases of liver or biliary tract disease.

METHODS

Prothrombin determination. The plasma prothrombin was measured quantitatively by a modified Quick method which has been described elsewhere (9). The prothrombin time was determined on 0.1 cc. portions of oxalated plasma in clean dry 100 by 13 mm. test tubes in a water bath at 37.5 C. No plasma showing gross lipemia was used. An excess of thromboplastin was supplied by the

addition of 0.1 cc. of a freshly prepared saline suspension of acetone dehydrated rabbit brain. One-tenth cc. of an optimal calcium chloride solution (9) was added with a blow pipette, and the time required for coagulation after the addition of calcium was observed with a stop watch. With optimal recalcification the normal prothrombin time was 10 seconds (equivalent to 100 per cent). The prothrombin time was converted to the percentage concentration of plasma prothrombin with the aid of a graph previously described (9). The prothrombin was determined on normal plasma on each occasion to assure proper activity of the thromboplastin and the absence of calcium contamination of the glassware. All prothrombin determinations were done by the authors. In most cases the test was performed three times per week, but in many instances it was done daily.

Vitamin K. The source of vitamin K used in a majority of the present observations was a preparation of dried young cereal plants (oats and wheat) in powder form, marketed under the name of Cerophyl.² A single allotment was used throughout the present studies. It was stored away from light in an ice box kept at 4 C. Elvehjem (17) assayed this material at 3-month intervals and found on each occasion that it protected chicks from hemorrhagic manifestations when added to a vitamin K free diet in the proportion of 1:400. Cerophyl contains other vitamins, namely carotene, thiamine, ascorbic acid and riboflavin. With the possible exception of carotene, these are present in such small amounts that it is unlikely that they would have significant effects.

The powdered preparation was mixed with milk, fruit juice or tomato juice and the suspension administered three times daily with meals. The total daily dose varied with the individual patient from 27 to 54 cc. These amounts were equivalent to from 12 to 24 grams. Concomitantly from 2 to 4 grams of animal bile salts were always given to aid in the absorption of the fat soluble vitamin (18). The bile salt preparation used was Bilron³ which was administered in 5 grain capsules. Experience in this clinic indicates that these dosages are greater than the minimal requirements for the control of the hemorrhagic diathesis in the usual case with obstructive jaundice.

In a few instances preparations of vitamin K other than powdered Cerophyl were employed. Five cases were

¹ This study was aided in part by a grant from the Wisconsin Alumni Research Foundation.

² The Cerophyl employed was generously supplied by the Cerophyl Laboratories, Kansas City Mo.

³ Eli Lilly and Company Indianapolis, Ind.

treated with 3 grams of a hexane extract of Cerophyl daily. This concentrate was sixteen times more potent by weight than the original material (17). Three patients were treated with 16 grams of a commercial alfalfa concentrate⁴ daily.

The vitamin K preparation and bile salts were administered orally to all subjects. Occasionally the medication was not well tolerated and caused nausea and vomiting. The patients were closely observed and if the medication was not retained it was repeated. In such individuals it was sometimes necessary to give the medication through a duodenal tube. In some patients, who could take nothing by mouth for several days after operation, continuation of the therapy was achieved by administration of the material through a duodenal tube which was inserted at the time of surgery.

Material studied. One hundred and thirty-six consecutive patients with liver or biliary tract disease were studied. All of the patients were adults except for 3 infants. Each patient was hospitalized during the period of observation. The diet in most instances was characterized by its low fat and high carbohydrate content. One hundred and five individuals in this group had various types of abdominal surgery performed.

The diagnosis was definitely established in the majority of these patients by histopathological studies. In 15 cases biopsy of the liver was done at the time of an exploratory operation. Complete autopsy was performed on 8 of the 10 cases who died. The icterus index, quantitative urobilinogen studies on the urine, stool examinations and blood studies were done routinely. The serum albumin and globulin were determined in all cases where hepatic damage was suspected. Additionally, the galactose tolerance test, hippuric acid test (19), and the cephalin-cholesterol test (20) were frequently employed.

RESULTS

Incidence of a reduction of prothrombin. The percentage concentration of plasma prothrombin was reduced below 100 in 64 cases, or 47 per cent of the 136 patients with liver or biliary tract disease studied. The remaining 72 patients had a normal plasma prothrombin on repeated determinations. There was no correlation between the intensity or duration of jaundice and the prothrombin value. Table I shows the number of patients with normal and reduced prothrombin in relation to the primary diagnosis.

Analysis of patients with normal prothrombin. None of the 72 individuals in whom a normal plasma prothrombin was repeatedly demonstrated showed evidence of a hemorrhagic diathesis. Forty-eight of these cases had abdominal surgery performed. Of this group, 20 patients received

TABLE I

Incidence of a reduction of prothrombin in 136 cases of liver or biliary tract disease

Diagnosis	Total number of cases	Number of cases with reduced prothrombin	Number of cases with normal prothrombin
Chronic cholecystitis with cholelithiasis	35	7	28
Chronic cholecystitis without cholelithiasis	19	1	18
Atrophic cirrhosis of liver	12	0	12
Acute hepatitis (toxic)	9	7	2
Stone in common bile duct	7	6	1
Obstructive biliary cirrhosis	6	6	0
Metastatic carcinoma of liver	6	3	3
Acute cholecystitis	6	0	6
Stricture of common bile duct	5	4	1
Carcinoma of head of pancreas	6	5	1
Acute catarrhal jaundice	5	1	4
Abscess of liver	3	2	1
External biliary fistula	3	3	0
Syphilitic cirrhosis of liver	3	3	0
Congenital absence of bile ducts	3	1	2
Banti's syndrome	3	1	2
Chronic catarrhal jaundice	2	0	2
Carcinoma of gall bladder	1	1	0
Actinomycosis of liver	1	1	0
Hemochromatosis	1	1	0
Totals	136	64	72

vitamin K and bile salts both before and after operation, while the remainder were untreated. No difference was observed in the course of the treated and untreated group. Three of this group of 72 patients died from causes unrelated to their biliary tract disease.

The remainder of this communication will be limited to a report of the observations on the 64 patients who presented a reduction in plasma prothrombin.

Analysis of patients with reduced prothrombin who were treated. Forty-six patients of the group of 64 with reduced plasma prothrombin were adequately treated with vitamin K and bile salts. The remainder of this group were selected as controls and received no treatment.

The effects of vitamin K and bile salt therapy on the prothrombin values in the 46 individuals with a deficiency of this coagulation factor were not uniform. Twenty-eight cases showed an increase of the prothrombin to normal or approximately normal values. Table II gives the diagnosis, prothrombin percentage before and after treatment, and the number of days of therapy required to produce the satisfactory response in these cases. The rate of prothrombin formation was quite variable. In most instances the increase in prothrombin was characterized by a regular progression in the percentage concentration. Al-

⁴ "Klotogen," Abbott Laboratories, North Chicago, Ill.

TABLE II

Effect of vitamin K and bile salts on the plasma prothrombin in 28 jaundiced patients with reduced prothrombin

Diagnosis	Prothrombin before treatment	Prothrombin after treatment	Time required for prothrombin response
	per cent	per cent	days
Chronic cholecystitis with cholelithiasis	55	100	9
Chronic cholecystitis with cholelithiasis	70	100	8
Chronic cholecystitis with cholelithiasis	68	100	4
Chronic cholecystitis with cholelithiasis	60	100	9
Chronic cholecystitis with cholelithiasis	50	100	7
Chronic cholecystitis with cholelithiasis	35	80	5
Stones in common bile duct	25	100	6
Stones in common bile duct	50	100	3
Stones in common bile duct	30	100	3
Stones in common bile duct	60	100	3
Obstruction of common bile duct (lymph node)	35	100	6
Stricture of common bile duct (adhesions)	30*	100	6
Stricture of common bile duct (adhesions)	5	70	10
Stricture of common bile duct (adhesions)	30	100	11
External biliary fistula	18	80	5
External biliary fistula	20	100	5
External biliary fistula	18	100	15
Congenital absence of bile ducts	18	100	8
Congenital absence of bile ducts	23	100	4
Carcinoma of head of pancreas	17	100	3
Carcinoma of head of pancreas	20	80	7
Obstructive biliary cirrhosis	45	100	7
Obstructive biliary cirrhosis	28*	100	17
Metastatic carcinoma of liver	25	100	6
Metastatic carcinoma of liver	30	80	6
Acute hepatitis (toxic)	48	100	10
Carcinoma of gall bladder	17	70	2
Abscess of liver	40	80	10

* Hemorrhage.

though an increase was usually observed within 24 hours after treatment was started the normal value was ordinarily not attained until several days later. These findings are similar to those recently reported by Smith, Ziffren, Owen and Hoffman (21) and are less dramatic than the prompt responses reported in the early literature. Figure 1 shows the rise in prothrombin which

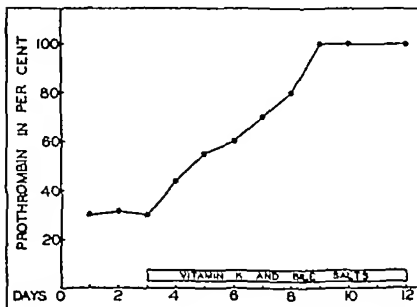


FIG. 1 EFFECT OF THE ORAL ADMINISTRATION OF VITAMIN K AND BILE SALTS ON THE PLASMA PROTHROMBIN IN A PATIENT WITH OBSTRUCTIVE JAUNDICE

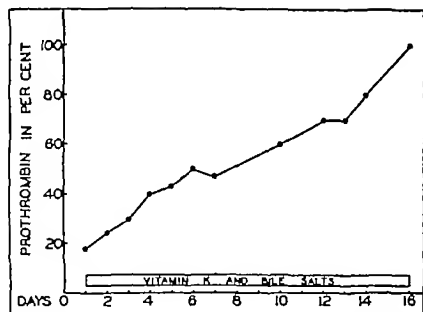


FIG. 2 EFFECT OF THE ORAL ADMINISTRATION OF VITAMIN K AND BILE SALTS ON THE PLASMA PROTHROMBIN IN A PATIENT WITH A CHRONIC EXTERNAL BILIARY FISTULA

followed the administration of vitamin K and bile salts to an individual with obstructive jaundice due to stones in the common bile duct. Figure 2 shows the improvement in prothrombin which followed similar therapy in a patient with an external biliary fistula of one and a half years duration. Figure 3 presents data from an indi-

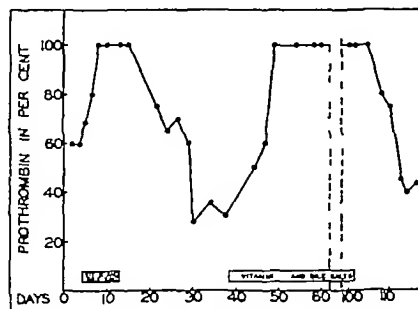


FIG. 3 EFFECT OF THE ORAL ADMINISTRATION OF VITAMIN K AND BILE SALTS AND THEIR WITHDRAWAL ON THE PLASMA PROTHROMBIN IN A PATIENT WITH OBSTRUCTIVE JAUNDICE

vidual with long standing obstructive jaundice due to adhesions about the common bile duct. This figure clearly shows the return of the prothrombin to normal on two occasions coincident with treatment and the fall to subnormal levels following withdrawal of therapy. Such data furnish strong

TABLE III

Lack of effect of vitamin K and bile salts on the plasma prothrombin in 18 patients with reduced prothrombin

Diagnosis	Prothrombin before treatment	Prothrombin after treatment	Duration of treatment
	per cent	per cent	days
Atrophic cirrhosis of liver	55	40	27
Atrophic cirrhosis of liver	70	50	24
Atrophic cirrhosis of liver	60	60	14
Atrophic cirrhosis of liver	50	50	9
Atrophic cirrhosis of liver	28	12*	21
Atrophic cirrhosis of liver	47	50	14
Atrophic cirrhosis of liver	45	35	16
Obstructive biliary cirrhosis	45	9*	15
Obstructive biliary cirrhosis	40	15*	17
Obstructive biliary cirrhosis	30	5*	18
Stone in common bile duct	20	30	8
Stone in common bile duct	80	20*	12
Carcinoma of head of pancreas	40	32	25
Carcinoma of head of pancreas	80	10*	14
Multiple abscesses of liver	70	22*	22
Metastatic carcinoma of liver	32	40	15
Congenital absence of bile ducts	50	50	25
Syphilitic cirrhosis of liver	70	70	15

* Hemorrhage

evidence that the vitamin K and bile salts were in some way responsible for the improvement noted

Eighteen of the 46 patients with a prothrombin deficiency who were treated showed no significant increase in prothrombin and thus may be classed as failures. Table III gives the diagnosis, prothrombin percentage before and after treatment and the number of days that the therapy was administered in these cases. These data show that not only was there no increase of prothrombin, but in some instances the treatment was accompanied by an actual reduction. Comparison of Tables II and III shows that the duration of therapy was greater in the group of 18 failures than in the group of 28 successes. In the 18 cases where there was no increase of prothrombin with treatment, the dosage of vitamin K and bile salts was often larger than in the group with good responses. If there was no improvement in the prothrombin value after several days of treatment the dosage was usually doubled. Figure 4 shows the failure of response of the prothrombin to treatment in a case with advanced atrophic cirrhosis of the liver. This figure is typical of the data obtained on the other patients with atrophic cirrhosis of the liver. In 5 surgical

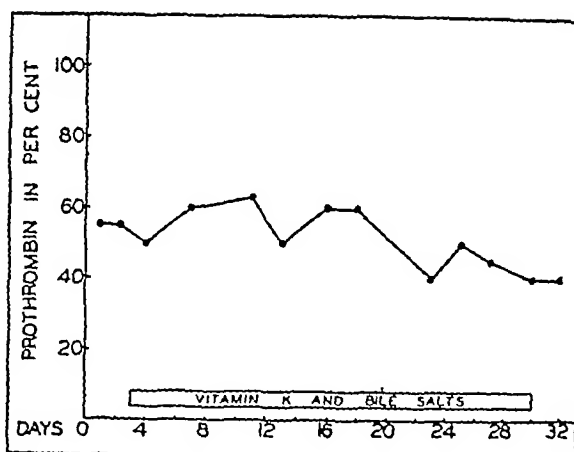


FIG 4 LACK OF EFFECT OF THE ORAL ADMINISTRATION OF VITAMIN K AND BILE SALTS ON THE PLASMA PROTHROMBIN IN A PATIENT WITH ADVANCED ATROPHIC CIRRHOSIS OF THE LIVER

cases, the therapeutic failure occurred during the postoperative period. In each of these 5 patients the prothrombin decreased to a low level and hemorrhage occurred in spite of the continuation of therapy. Figure 5 gives the data on these patients.

Analysis of patients with reduced prothrombin who were not treated. Eighteen patients of the group of 64 with reduced plasma prothrombin served as controls and received no vitamin K or bile salts. In no case was the period of observation less than 12 days. Eleven of these patients, 7 of whom underwent surgery, showed no significant change in their prothrombin value. One other untreated patient showed a return of the prothrombin to normal following surgical relief of obstruction to the common bile duct (Figure 6). The one fatality in this group was an individual with obstructive jaundice due to a carcinoma of the pancreas in whom the prothrombin was persistently below 25 per cent. This patient became comatose and died after 12 days of observation. Two large subdural hematomata were found at autopsy. The remaining 5 untreated patients suffered from an acute toxic hepatitis. All showed a progressive reduction in prothrombin as the severity of the disease increased. This was followed by a gradual return of the prothrombin to normal with recovery from the hepatitis. Figure 7 presents data on one of these cases in whom the hepatitis followed a hysterectomy under

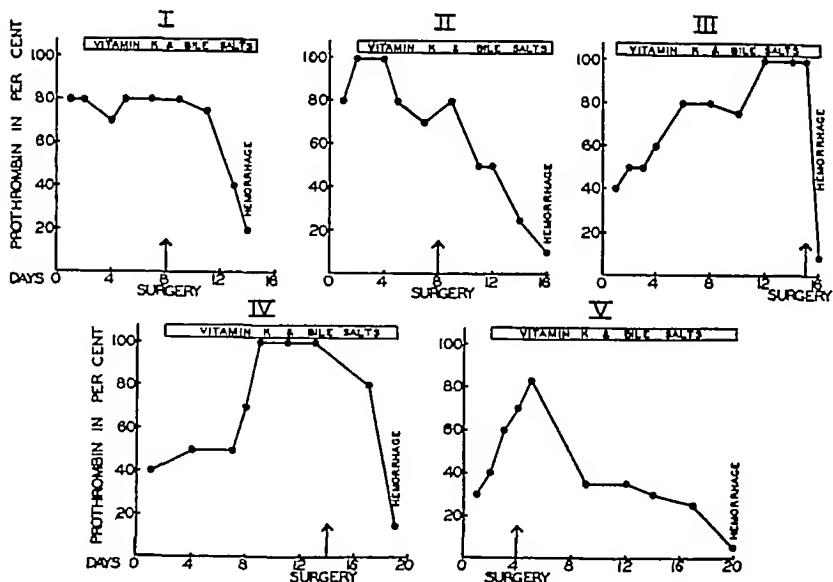


FIG. 5 POSTOPERATIVE DECREASE IN PLASMA PROTHROMBIN WITH HEMORRHAGE, IN 5 PATIENTS WITH OBSTRUCTIVE BILIARY CIRRHOSIS

inhalation anesthesia. The changes in the prothrombin and icterus index shown in Figure 7 are typical of those observed in the other patients with acute hepatitis.

Analysis of patients who bled Ten of the 64

patients with a reduction of prothrombin had definite abnormal bleeding. Seven of these 10 patients died as a result of hemorrhage. The data in Table IV show that the bleeding tendency in each case was associated with a marked de

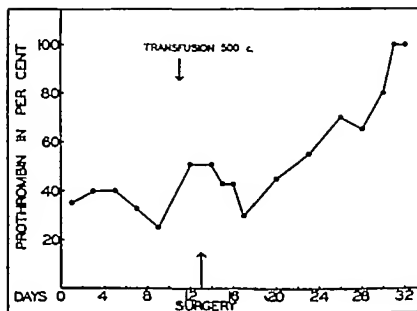


FIG. 6. CHANGES IN THE PLASMA PROTHROMBIN FOLLOWING SURGICAL RELIEF OF OBSTRUCTION TO THE COMMON BILE DUCT

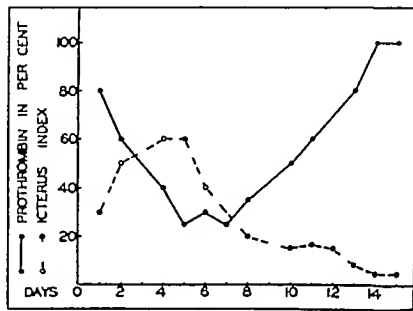


FIG. 7. CHANGES IN THE PLASMA PROTHROMBIN AND ICTERUS INDEX DURING THE COURSE OF AN ACUTE TOXIC HEPATITIS

TABLE IV

Prothrombin values and clinical notes on 10 jaundiced patients who bled

Diagnosis	Adequate vitamin K and bile salts prior to hemorrhage	Prothrombin at time of hemorrhage	Clinical notes
		per cent	
Stones in common bile duct with obstructive biliary cirrhosis (Case I, Figure 5)	Yes	20	Massive intra abdominal hemorrhage on 6th postoperative day Evisceration. Death. Autopsy
Carcinoma of head of pancreas with obstructive biliary cirrhosis (Case II, Figure 5)	Yes	10	Massive intra-abdominal hemorrhage on 8th postoperative day Death. Autopsy
Advanced obstructive biliary cirrhosis (Case III, Figure 5)	Yes	9	Massive intra abdominal hemorrhage on 1st postoperative day Death. Autopsy
Advanced obstructive biliary cirrhosis (Case IV, Figure 5)	Yes	15	Intra abdominal hemorrhage on 4th postoperative day Death. Autopsy
Advanced obstructive biliary cirrhosis (Case V Figure 5)	Yes	5	Hemorrhage into abdominal cavity on 18th postoperative day
Obstructive jaundice due to carcinoma of head of pancreas	No	16	Two large subdural hematomata following slight head injury Death. Autopsy
Atrophic cirrhosis of liver with subacute hepatitis	Yes	12	Bleeding from abdominal paracentesis wound and from gastrointestinal tract. Death. Autopsy
Obstructive jaundice due to adhesions about common bile duct	No	30	Bleeding from the gums.
Advanced obstructive biliary cirrhosis	No	28	Epistaxis. Severe bleeding from the gums.
Multiple abscesses of liver	Yes	22	Intra-abdominal hemorrhage. Death. Autopsy

crease in the plasma prothrombin This table also shows that the hemorrhage occurred in 7 of the 10 cases in spite of the administration of vitamin K and bile salts One other patient, with a prothrombin of 60 per cent, bled from the abdominal wound during the second and third postoperative days This case was not included in Table IV since exploration of the incision revealed the source of hemorrhage to be a single unligated vessel In this case the cause of bleeding was interpreted as mechanical rather than being due to a coagulation defect from prothrombin deficiency

DISCUSSION

There is general agreement that normal human plasma contains an excess of prothrombin which under healthy conditions shows only slight fluctuations A decrease in prothrombin occurs prin-

cipally in jaundiced individuals Quick (7), however, states that relatively few jaundiced patients exhibit such a deficiency In 136 cases with liver or biliary tract disease observed in the present investigation, a reduction in the prothrombin was demonstrated in 64 (47 per cent) This figure coincides rather closely with the incidence of 40 per cent in 43 patients with obstructive jaundice reported by Rhoads (13)

The present observations confirm previous studies which indicate that the prothrombin determination is a satisfactory test for the detection of a hemorrhagic diathesis in jaundiced individuals The need for such a prognostic test is obvious since current figures (8) indicate that cholemic bleeding has accounted for about 50 per cent of the mortality accompanying surgical intervention in jaundiced patients In the present series of 136 cases having jaundice, 10 showed abnormal bleeding Each of these 10 cases with hemorrhage had a prothrombin of 30 per cent or below Fifteen other cases with this degree of prothrombin deficiency showed no hemorrhagic tendency The establishment of a "critical level" of prothrombin below which cholemic bleeding may be anticipated is dangerous because of the false sense of security it may give in the evaluation of certain borderline cases These data suggest, however, that any patient with a prothrombin concentration of 30 per cent or below should be considered a potential bleeder

Vitamin K and bile salts have been used in the treatment and prevention of cholemic bleeding because of the beneficial effect on prothrombin formation Many of the clinical reports have been presented with enthusiasm and, in general, received with optimism Few cases have been recorded in which this new therapy has failed to restore the prothrombin to normal (13) The present studies clearly show that in the majority of jaundiced patients with prothrombin deficiency the administration of vitamin K with bile salts corrects the defect in this coagulation factor These studies also show, however, that there is a lack of a favorable response in a significant number of cases The 18 failures noted in this investigation cannot be ignored

The explanation for the failure of the prothrombin to respond to treatment in 18 patients seems to be of special interest Inaccuracies in the

quantitative determination of prothrombin cannot be blamed because the results were not evaluated entirely by this criterion. In 7 of these patients (Table III) persistence of a low level of prothrombin was accompanied by alarming and sometimes fatal hemorrhage. The dosage of vitamin K and bile salts appeared to be adequate since the amount and duration of therapy were greater in the 18 failures than in the 28 patients who responded satisfactorily. The administration of larger amounts of the crude preparation of vitamin K was not practical. The data in Tables II and III and the other laboratory studies on these 46 patients suggest that the essential difference between the therapeutic successes and failures was the state of the liver function. The group of 18 so-called failures was composed of patients in whom liver function tests or histopathological studies or both revealed extensive damage to the liver parenchyma. Similar studies showed no indication that hepatic damage of this degree was present in the group of 28 patients who responded satisfactorily. Relatively little attention has been paid to the functional integrity of the liver in evaluation of the prothrombin response to vitamin K and bile salt therapy. Warner (22) and Butt, Snell and Osterberg (8) have encountered an occasional patient with severe hepatic damage who did not present the usual prompt improvement of prothrombin following the administration of vitamin K and bile salts. The present studies indicate that vitamin K and bile salts are commonly ineffective in correcting a prothrombin deficiency if extensive hepatic damage is present.

Recent experiments indicate that the liver is the site of formation of prothrombin (2, 23, 24, 25). The observations on the prothrombin fluctuations during acute hepatitis (Figure 7) add further support, of a clinical nature, to this conclusion. These data also suggest that in the absence of biliary obstruction, external biliary fistula or an abnormal intestinal absorptive surface the plasma prothrombin concentration is a measure of liver function.

Studies by Butt, Snell and Osterberg (8) and others have shown that a decrease in prothrombin is not uncommon after operations on jaundiced individuals. Stewart (26) observed that an aver-

age postoperative decrease in prothrombin of 23 per cent occurred in 19 cases with obstructive jaundice. Butt, Snell and Osterberg have emphasized the necessity for continued administration of vitamin K and bile salts as well as other treatment known to be of value in the restoration of normal hepatic function during the postoperative period. The present observations also show that a decrease of prothrombin is not uncommon after surgery and suggest that this drop is especially likely to occur if significant hepatic damage is present.

The mechanism by which vitamin K affects prothrombin has not been fully explained. It has been presumed that vitamin K is a necessary building stone for the formation of prothrombin. It has not, however, been proven that vitamin K is essential in the diet of human beings. The nature and mode of action of prothrombin are not known. Patek and Taylor (27) have described it as a physiologic complex known only by its capacity to form thrombin. The mode of action of vitamin K will not be fully understood until further studies are made with the purer preparations of the vitamin now available, and until more is learned about the nature of prothrombin and the part it plays in blood coagulation.

CONCLUSIONS

1. In 136 consecutive cases of liver or biliary tract disease the incidence of a reduction in plasma prothrombin below normal was 47 per cent. Intrinsic liver disease was a frequent cause of the prothrombin deficiency.

2. A marked reduction of the plasma prothrombin was present in each of 10 individuals who exhibited abnormal bleeding. The data suggest that hemorrhage should be anticipated when the prothrombin concentration is 30 per cent or below.

3. The effect of the oral administration of vitamin K and bile salts on the prothrombin in 46 jaundiced patients with a reduction in this coagulation factor was not uniform. Twenty-eight patients showed a satisfactory increase in prothrombin while 18 patients showed no improvement.

4. The failure of vitamin K and bile salts to produce an increase in the prothrombin in certain

cases with jaundice is often due to the presence of extensive hepatic damage

5 The decrease in prothrombin which is not uncommon after surgical intervention in jaundiced patients is especially likely to occur if hepatic damage is present

6 The present studies suggest that in the absence of obstructive jaundice, external biliary fistula, or an abnormal intestinal absorptive surface, the plasma prothrombin concentration serves as a measure of liver function

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OBSERVATIONS ON THE SERUM CHOLESTEROL IN ACUTE INFECTIONS AS RECORDED DURING AND AFTER PNEUMONIA¹

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In a previous report (1) the relative stability of the serum cholesterol level was demonstrated in normal subjects over long periods of time. The cholesterol values were not altered significantly by the feeding of diets high or low in fat or cholesterol or by the ingestion of potassium iodide. However the administration of thyroid extract produced a sharp fall in serum cholesterol which was followed by a rise above normal when thyroid feeding was discontinued.

Acute infection is also known to produce a hypocholesterolemia. This was shown a number of years ago by Denis (2) and Kipp (3) and by numerous workers since. The behavior of the serum cholesterol during convalescence has received little attention. Stoesser and McQuarrie (4) and more recently Stoesser (5) reported that a plasma cholesterol determination made in infants or children convalescent from an acute infection was higher than one obtained at the height of the febrile episode. However there have been no reports in which repeated determinations were made on the same individual during and for a protracted period after the acute infection. Accordingly the present study was undertaken in an attempt to demonstrate what happens to the serum cholesterol of individuals who develop an acute infection—an infection which was in these instances pneumonia.

PLAN OF STUDY

Observations were made on 19 patients varying in age between 12 and 52 years who were admitted to the hospital with a diagnosis of pneumonia. There were 12 males and 7 females of whom 16 were white and 3 were colored. In the sputum studies on the 19 patients the pneumo-

coccus type was identified in 9 and the hemolytic streptococcus was found in 3. Five patients were treated with specific serum, 4 with sulfanilamide, and 1 with optochin. An initial serum cholesterol was determined using the method of Bloor, Pelkan and Allen (6) from blood obtained within 48 hours of the patient's entry to the hospital. Serum cholesterol determinations were made twice weekly thereafter while the patients remained in the hospital. After discharge the patients were seen at weekly, bi-weekly or monthly intervals for periods ranging from 96 to 520 days. The total number of tests performed was 418, averaging 22 for each patient.

Basal metabolism estimations were made at frequent intervals after the fever subsided. In addition to the usual laboratory procedures serum protein and bromsulphalein dye retention determinations were made on most of the patients.

Serum cholesterol level during febrile period

During the febrile period of pneumonia there is a hypocholesterolemia. At the lowest point of each individual curve which was observed usually on the second to sixth day of the disease the total serum cholesterol ranged between 86 and 206 mgm per 100 cc. (Table I). At this time a reduction in cholesterol ester was apparent in the 6 patients in whom this fraction was determined. In 1 case the lowest value for the total serum cholesterol was not reached until the twenty-second day. The course of this patient was complicated by the development of active rheumatic carditis subsequent to pneumonia due to a hemolytic streptococcus.

Serum cholesterol level during convalescence

As soon as the fever subsided the cholesterol value in each patient rose, and for

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TABLE I
 Serum cholesterol values during and after pneumonia

Name	Sex	Age	Pneumonia		Days of fever over 102°	Treatment	Total days observed	Number of cholesterol determinations	Serum cholesterol									Elevation of serum cholesterol	
			Type	Location					Low			High			"Normal"			Ext-	Duration
									Total	Ester	Day	Total	Ester	Day	Total	Ester	Day		
J. M.	F	17	Pneumococcus IV	L. L. L.	5	Sulfanilamide	168	31	mgm.	per cent		mgm.	per cent		mgm.	per cent		per cent	days
M. G.	M	25		R. M. L.	9		210	31	91		10	320		72	230		91	39	60
G. S.	M	24	Pneumococcus IV	L. L. L.	8		192	18	140		5	274		39	225		60	23	39
J. A.	M	28		R. L. L.	3	Serum	112	21	121		2	297		50	310		69	41	50
L. L.	F	38	Pneumococcus I	L. L. L.	4		120	23	182	36	4	332	84	37	274	82	85	25	30
R. D.	F	20		L. L. L.	2		117	26	101		2	285		25	200		87	42	63
J. S.	M	16		Bronchi	3	Sulfanilamide	125	9	188		3	263		46	185		76	58	60
M. B.	M	12	Hemolytic streptococcus	Bronchi	5		96	17	206		4	270		27	200		70	35	35
D. C.	M	15		R. U. L.	4	Serum	506	43	123	48	6	450	68	76	200	83	170	125	110
A. N.	M	52	Pneumococcus II	L. L. L.	7		187	26	132	54	3	344	86	33	235	70	83	42	61
C. P.	F	28	Pneumococcus IV	R. U. L.	5		336	26	166	42	5	299	78	44	210	79	78	42	65
L. B.	M	17		L. L. L.	5	Sulfanilamide	124	16	157		3	277		31	180		47	53	20
V. L.	F	53		R. L. L.	6		119	12	161		5	263		44	220		65	20	31
W. M.	M	24	Pneumococcus VII	L. L. L.	2		156	22	176		4	316		39	266		63	20	32
J. S.	M	34	Pneumococcus I	R. U. L.	5	Serum	112	20	86		3	261		30	235		90	11	30
M. D. a	M	21	Pneumococcus VII	L. U. L.	6		330	29	125	20	7	358	73	56	235	76	140	52	57
M. H.	M	15	Hemolytic streptococcus	L. L. L.	14		510	39	96		22	239	87	68	200	84	150	20	80
M. D. b	F	44	Hemolytic streptococcus	L. L. L.	12	Sulfanilamide	105	12	125		4	295		10	255		85	17	47
C. R.	M	52	Pneumococcus IV	R. U. L.	7		520	36	136		10	255		28	225		140	13	92
						Average	217	22						40			93	38	52

time during convalescence strikingly wide fluctuations occurred in the cholesterol level (Figure 1). In general, there was a hypercholesterolemia due to an increase in both the ester and free fractions. The serum cholesterol of each of the 19 patients reached a peak during convalescence between the tenth and seventy-sixth days, usually about the fortieth day. The increase in serum cholesterol over the level that was subsequently found to be "normal" for each of the patients varied from 26 to 250 mgm per 100 cc, with an average rise of 82 mgm per 100 cc. The percentage of increase over the normal value varied from 11 to 125 per cent, with an average rise of 38 per cent. The elevation of the serum cholesterol persisted from 20 to 110 days, with an average of 52 days (Table I). Although the serum cholesterol fluctuated considerably during this period, the general pattern of response was characterized by a rise to a peak, with a subsequent fall toward normal (Figure 1).

Normal serum cholesterol level

The serum cholesterol reached the level designated as normal from 47 to 170 days after the onset of the illness (Table I). At this point fluctuations in the cholesterol level disappeared and

repeated determinations revealed values which were constant for the individual. Previous work by the authors (1) has shown the serum cholesterol to be remarkably constant in normal subjects over long periods of time. The results obtained in this study during the "normal" period which varied from 30 to 380 days, averaging 123 days, confirm this finding. Figure 1 demonstrates this observation in 6 of the patients. In patient D. C. during the 336 days of the normal period the serum cholesterol deviated only 7 mgm from the mean. In patient C. P. the serum cholesterol varied only 8 mgm from the mean during the normal period of 258 days.

DISCUSSION

From the foregoing it is evident that during pneumonia there is a decrease in the serum cholesterol level. After the infection subsides and the fever disappears there follows characteristically a rise above the level later found to be normal for the individual, with a marked instability of the serum cholesterol values which may persist for weeks or months before the stabilized level assumed to be normal is reached.

The mechanism producing this alteration in serum cholesterol is not clear. Because of the simi-

SERUM CHOLESTEROL DURING AND AFTER PNEUMONIA

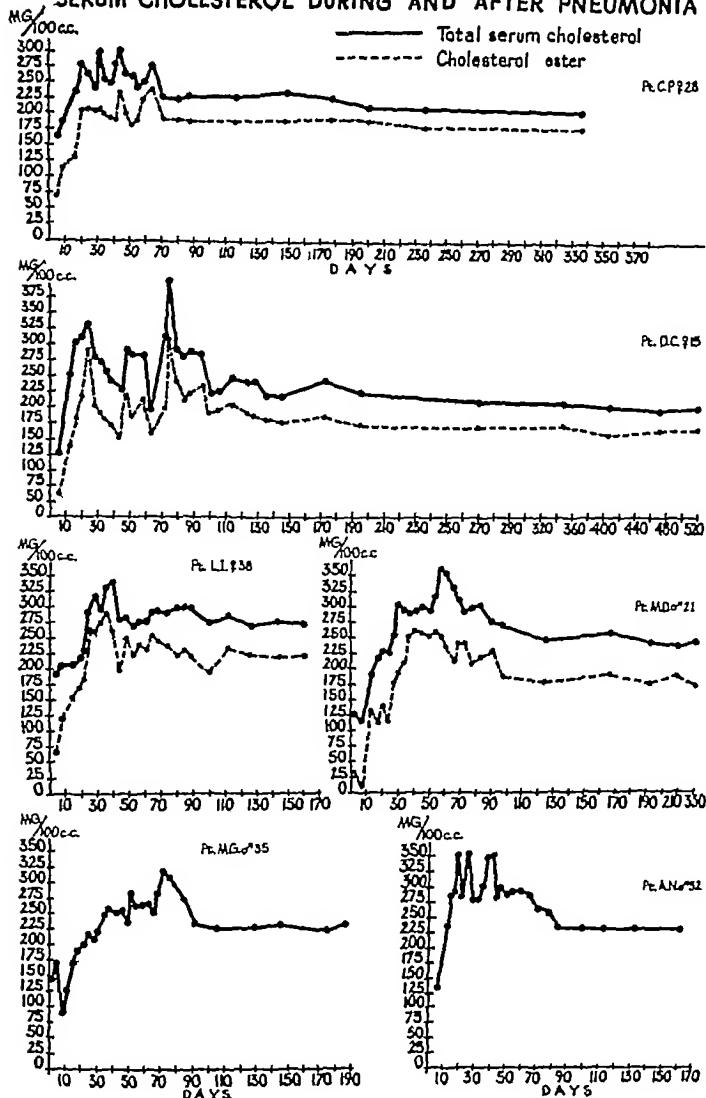


FIG 1. ILLUSTRATIVE CURVES SHOWING THE DEPRESSION OF THE SERUM CHOLESTEROL DURING PNEUMONIA, THE RISE TO ABNORMALLY HIGH LEVELS ASSOCIATED WITH MARKED FLUCTUATIONS DURING CONVALESCENCE, AND THE STABILITY CHARACTERISTIC OF NORMAL WELL-BEING

area during January, February, and March, the attack rate was 6 per cent, which was considerably lower than the rates observed in the two institutional epidemics. In both institutions the epidemics were explosive in character and had sharp peaks which occurred during periods of 2 or 3 days, while the entire episodes ended within periods of less than 2 weeks. Table III reveals that Epidemic 3 spread over a period of approximately 2½ months, with a peak during the 15-day interval from February 5 to 18, inclusive. During the month of April 1939 only 2 cases of febrile upper respiratory disease were observed in the Yorktown Heights area. As this was the same incidence that had been observed in April 1938, it was concluded that the epidemic had ended during the latter part of March.

There was no significant geographical distribution of the cases in Epidemic 3. Forty-two occurred as single cases in a family, while the remaining 41 were distributed among 14 families with 2 to 7 cases in each. Distribution by age and sex followed closely the age and sex distribution of the total population under observation, with the exception that no cases were noted in children under 4 years of age. This discrepancy might be due to the fact that the disease was relatively mild, with symptoms largely of a subjective nature and, therefore, not readily recognized in infants and small children.

METHODS

Throat washings Sixty-five throat washings were obtained from representative patients in the four epidemics who had clinical symptoms considered to be consistent with the diagnosis of epidemic influenza. Forty-six throat washings were taken from patients whose symptoms were considered not to be consistent with epidemic influenza. The latter group comprised 18 cases of common cold, 9 cases of sporadic grippe, 11 cases of so-called "atypical pneumonia," and 8 cases of pneumococcus pneumonia, all of which occurred between October 1 and December 31, 1938. A few cubic centimeters of either sterile beef infusion broth or equal parts of broth and sterile saline were dropped into the nostrils of each patient, and after this approximately 30 cc. of broth or broth-saline were gargled. The throat washings were frozen in solid carbon dioxide within 5 minutes of the time when they were taken, and they were transported to the laboratory in the frozen state. During the intervals between the time that the throat washings were obtained and their inoculation into ferrets they were stored in a low temperature cabinet (13) at -76°C . The washings were taken at various intervals after the onset of the disease, and in the case of epidemic influenza, washings on each day from the 1st to the 8th were studied.

Blood specimens Two or more blood specimens were taken from each of 146 patients in the four epidemics, as well as from 117 persons who had either no symptoms or noninfluenzal respiratory disease. The latter group comprised 33 persons who had had intimate contact with cases of epidemic influenza, 56 with cases of common cold, 19 with cases of sporadic grippe, 5 with cases of

so-called atypical pneumonia, and 4 with cases of pneumococcus pneumonia. The acute-phase blood specimens were taken at various intervals after the onset of the disease, and specimens from the 1st to the 10th day have been studied. Convalescent specimens were taken from the 10th to the 66th day after onset. In the case of Epidemic 4 specimens were taken from each of 20 patients at weekly intervals for 4 weeks. Serum was separated from the clot in the usual way and was stored at 4°C .

Inoculation of ferrets Two cubic centimeters of the unfiltered throat washings were routinely inoculated intranasally into a ferret under ether anesthesia. Ferret temperatures were taken twice daily, and any abnormal nasal signs, respiratory symptoms or decrease in food consumption were noted. Ferrets were considered to show evidence of experimental influenza when their temperature exceeded 103.9°F on the 2nd, 3rd, or 4th day after inoculation and when they simultaneously manifested signs of nasal infection, increase in respiratory rate, and a decrease in appetite. Serial passage in ferrets was carried out routinely at an interval of 4 to 5 days. For the purpose of passage 2 cc. of a 20 per cent suspension of turbinate plus lung, or of lung alone, were inoculated intranasally into ferrets anesthetized with ether. The suspensions were routinely cultured for the presence of bacteria. When evidence was desired regarding immunity in an inoculated ferret, the animal was allowed to recover from infection and was bled on the 10th to the 12th day after inoculation. Neutralization tests were done in mice with the convalescent ferret serum.

Inoculation of mice When, by means of serial ferret passage, evidence had been obtained that a virus was present in the throat washings, attempts to adapt the virus to mice were initiated in many instances. For this purpose 0.05 cc. of unfiltered 20 per cent suspension of ferret turbinate plus lung, or of lung alone, was given intranasally to each of a group of young Swiss mice anesthetized with ether. Serial passage in mice was continued at an interval of 4 to 5 days, routinely using 0.05 cc. of unfiltered 20 per cent suspension of mouse lung. The suspensions were routinely cultured for the presence of bacteria.

Neutralization tests The exact details of the technique which has been used for the determination of the neutralizing titer of sera have been described previously (14). The sera were inactivated at 56°C for ½ hour. Acute and convalescent sera were studied simultaneously against one suspension of the PR8 strain of epidemic influenza virus. Serial fourfold dilutions of serum were made in 0.85 per cent NaCl. These dilutions were tested for their capacity to neutralize a constant amount of virus. In one-third of the tests a 10^{-4} dilution, or approximately 300 fifty per cent mortality doses, of virus was used. In the other two-thirds of the tests a 10^{-5} dilution, or approximately 3,000 fifty per cent mortality doses, of virus was used. Groups of either three or four Swiss mice were tested with each mixture of serum and virus and observed for a 10-day period. The survivors

were then killed and their lungs examined for the presence of pulmonary consolidation. The calculation of end points was done by the method of Reed and Muench (15). In all instances the fifty per cent mortality end point was chosen. In the various tables presented below all serum titers are expressed in terms of the neutralizing capacity against 3000 fifty per cent mortality doses of virus. In the case of those neutralization tests in which 300 fifty per cent mortality doses were used the recorded titers have been calculated by means of the formula $y = bx^a$ (14) on the basis that there is a linear relationship between the quantity of serum and the quantity of influenza virus neutralized. A significant increase in the neutralizing antibody titer of a convalescent serum has been taken to equal an increase in titer of 4 or more times over the acute serum titer.

Tests of antisera. Convalescent ferret sera were obtained 10 to 12 days after inoculation. Rabbit antisera were obtained 10 to 12 days after the intraperitoneal injection of either ferret or mouse-adapted virus. These antisera were studied in a manner identical to that described above.

Isolation of virus. One hundred and eleven throat washings have been tested for the presence of epidemic influenza virus by serial passage in ferrets. The throat washings were selected from 65 representative cases of epidemic influenza and from 46 cases of various acute noninfluenzal infections of the respiratory tract. The selection of throat washings was made entirely on a clinical basis without regard for the demonstration of a rise in antibodies against epidemic influenza virus in the convalescent sera of the patients from whom they were obtained. Three hundred and eighty eight ferrets were used in this study and each throat washing from both groups of cases was passed an average of 3.5 times in ferrets.

EXPERIMENTAL

The results of the serial ferret passage of the throat washings are summarized in Table IV. Twenty nine of the 65 throat washings from patients with clinical epidemic influenza produced what was considered to be experimental influenza in the ferret. In the case of 14 of these 29 strains of virus antisera obtained from various of the ferret passage series have contained neutralizing antibodies against the PR8 strain of epidemic influenza virus. In the case of the remaining 15 strains of virus the studies are not yet completed. Despite the fact that serial ferret passage was carried out with the throat washings from 65 cases of epidemic influenza, only 45 per cent were capable of producing experimental ferret influenza. Evidence will be presented subsequently which indicates that at least 93 per cent of the patients from whom these 65 throat washings were taken

TABLE IV

Results of serial ferret passage of throat washings from persons with various acute infections of the respiratory tract

Clinical diagnosis	Number of throat washings	Number producing ferret influenza	Number of strains of virus proven	Percent producing ferret influenza
Epidemic influenza				
Epidemic 1	6	4	1	66
Epidemic 2	18	9	3	50
Epidemic 3	26	11	8	42
Epidemic 4	15	5	2	33
	65	29	14	45
Noninfluenzal respiratory disease				
Common cold	18	0	0	0
Sporadic grippé	9	1	1	11
Atypical pneumonia	11	0	0	0
Pneumococcus pneumoniae	8	0	0	0
	46	1	1	2

actually did have epidemic influenza. This relatively low recovery rate of virus is in contrast to the high recovery rates which have previously been published (9-12) for epidemics which occurred in 1936 to 1937.

From the 46 throat washings obtained from persons with noninfluenzal acute respiratory diseases only one strain of epidemic influenza virus was isolated.

This strain was obtained from a throat washing taken on November 11, 1938, from a technician in this laboratory who had symptoms of sporadic grippé. This individual had been working for over 2 months with various animal and tissue culture strains of epidemic influenza virus. Studies of blood specimens from this individual taken on the 1st day of the illness and 20 days later failed to show any evidence of an increase in titer of neutralizing antibodies against the PR8 strain of epidemic influenza virus.

It seems reasonable to suggest that the isolation of virus from this individual may represent an additional instance of a possible temporary carrier state unrelated to the clinical symptoms. One somewhat similar instance has been previously reported by Francis Magill Rickard, and Beck (9). In the case of the other 45 throat washings in the noninfluenzal group no evidence was obtained of the presence of influenza virus.

The 65 throat washings from cases of epidemic influenza were taken from the 1st to the 8th day

TABLE V

Results of serial ferret passage of throat washings taken at various intervals after onset of epidemic influenza

Number of days after onset	Number of throat washings	Number producing ferret influenza	Number of strains of virus proven	Per cent producing ferret influenza
1	14	6	5	42
2	19	11	3	58
3	14	7	4	50
4	7	2	1	28
5	2	1	1	50
6	5	1		20
7	2	1		50
8	2	0		0
	65	29	14	45

after onset. The results of the serial ferret passage of these various throat washings in relation to the time when they were obtained are shown in Table V.

It will be noted that experimental ferret influenza was produced by throat washings which were taken on each of the days from the 1st to the 7th after onset, and that the fact that the ferret disease was actually due to influenza virus infection had been proved in the case of throat washings taken on each of the days from the 1st to the 5th after onset of the disease. It appears also that there was no significant difference in the incidence with which experimental ferret influenza was produced by throat washings taken from the 1st to the 7th day after onset. It has previously been reported (11) that virus was isolated from one patient on the 1st, 2nd, 4th, and possibly the 7th day after onset.

Serial ferret passage, although laborious and time consuming, has been considered necessary with the throat washings obtained from the 1939 epidemics of influenza because of the frequency with which the first ferret inoculated failed to show an interpretable reaction. Even when the ferret reaction was definite enough to indicate infection by epidemic influenza virus, it was considerably less striking than has been the case in previous years (1, 12), and a diphasic fever was typically absent. Instead there was usually a single temperature peak which occurred either upon the 2nd, 3rd, or 4th day after inoculation, and the highest temperature seldom exceeded 104.5° F.

In the case of the 29 throat washings which

produced experimental influenza in the ferret, 6 produced no significant response in the first ferret inoculated, 5 of these produced a suggestive response in the second-passage ferret, but one did not produce recognizable disease in the ferret until the third passage. Even in the case of the 23 throat washings which produced some evidence of experimental influenza in the first ferret inoculated, the response in this animal was minimal and was interpreted with difficulty until additional ferret passages had been completed.

To illustrate the difficulties encountered in the study of throat washings obtained from epidemics in 1939, an analysis has been made of the results of *relatively prolonged serial ferret passage* with 21 of the 29 strains isolated this year. The results obtained in a total of 150 ferrets have been analyzed. An average of 7.1 passages in ferrets was made with each of these 21 strains. These results are presented in Table VI. It will be noted that, although evidence indicating the presence of epidemic influenza virus was obtained in the case of each of the 21 throat washings, only 15 of the first-passage ferrets showed significant responses. Eighty-five ferrets received unfiltered 20 per cent suspensions of ferret turbinate plus lung in the course of the serial passages, but only 53 gave evidence of the presence of epidemic influenza virus. In the case of 44 ferrets which received unfiltered 20 per cent suspension of ferret lung, only 24 developed fever and symptoms indicative of epidemic influenza virus infection. These results are presented as evidence that the ferret response has been inconstant with strains isolated in 1939. In 38 per cent of 150 ferrets

TABLE VI

Results of serial ferret passage of 21 throat washings known to contain epidemic influenza virus

Inoculum*	Number of ferrets	Number with influenza	Number with out influenza	Per cent with influenza
Unfiltered throat washings	21	15	6	72
20 per cent suspension ferret turbinate and lung	85	53	32	63
20 per cent suspension ferret lung	44	24	20	55
	150	92	58	62

* 2 cc.

which received 21 strains the reaction produced was not sufficiently definite to permit of a diagnosis of experimental ferret influenza. These results indicate also that even when consolidation was not present in the ferret lung, evidence for the presence of virus in the lung was obtained with almost the same frequency as when the turbinates also were tested. The fact that ferrets which did not show a significant response to the 1939 strains actually were infected by these strains was shown by neutralization tests on serum taken from them 10 to 12 days after inoculation.

Additional evidence for the relatively low ferret virulence of the various strains of virus which have been isolated in 1939 is given by the infrequency with which pulmonary consolidation was produced. Three of the most ferret virulent strains were selected for continuous passage. Strain 149 was passed through 23 ferrets and produced small areas of consolidation at the 12th 20th 21st, and 22nd passages but not in other passages. Strain 188 was passed through 25 ferrets and produced small areas of consolidation at the 9th 17th, and 22nd passages but not in other passages. Strain 399 was passed through 16 ferrets and produced small areas of pulmonary consolidation in the 7th 8th 12th 13th and 15th passage ferrets but not in any other ferrets.

Adaptation to mice. Nineteen attempts have been made to establish in mice 15 of the 29 strains which have been isolated in 1939. Each of these attempts was made after serial ferret passage of the virus. Ten of the attempts have been unsuccessful although serial passage in mice was continued. In nine instances the various virus strains have been successfully established in mice. The results of the serial mouse passages are shown in Table VII. It will be noted that in the ten unsuccessful attempts an average of only 2 ferret passages had been carried out prior to the inoculation of mice. An average of 6 serial passages in mice was made, and in no instance did the virus produce significant lesions in the mouse. In the case of the nine successful adaptation series an average of 4 serial ferret passages was made before the inoculation of mice and an average of 4 serial mouse passages was necessary before significant lung lesions were produced. As has previously been found by other workers (2, 16) mouse virulence gradually increased after adapta-

TABLE VII
Results of serial mouse passage of 15 strains of epidemic influenza following serial ferret passage

Strain number	Unsuccessful attempts to adapt virus to mice		Successful attempts to adapt virus to mice	
	Number of ferret passages	Number of mouse passages	Number of ferret passages	Number of mouse passages
134	2	2		
135	2	2		
149			5	4
188			4	8
206			2	4
208	3	3	2	5
210			4	4
233			10	3
236	3	5		
237	2	8		
241	2	10		
273			1	5
390			3	3
399	3	10	9	4
400	3	10		
237	1	8		
237	3	2		
Mean	2	6	4	4

tion. However in most instances the 1939 viruses have remained of relatively low virulence for mice with a fifty per cent mortality end point of only 10^{-3} or 10^{-4} . In only two instances have the viruses been sufficiently virulent to permit of dilutions to 10^{-5} and still produce fatal infections in mice.

Tissue culture. Eight separate attempts were made to cultivate 4 strains of the 1939 viruses in chick-embryo-Tyrodé tissue culture medium (17). Each of these strains had been well established in mice before tissue culture passage was instituted. In only three of the eight series did the strains remain active for more than 2 or 3 passages.

Antigenic analysis. Strains 149 and 188 which were obtained from Epidemics 1 and 2 respectively were selected for intensive antigenic study. Antisera against these strains and the PR8 and WS strains were prepared in both ferrets and rabbits. These sera were tested for their capacity to neutralize the homologous and the heterologous viruses. Both of the 1939 strains which have been carefully studied appeared to be closely related antigenically although they did not seem to be absolutely identical. By means of rabbit anti-serum neutralization tests both strains were quite different from either the PR8 or the WS strain. However when homologous convalescent ferret

TABLE VIII

Results of neutralization tests with epidemic influenza virus on two blood specimens from each of 60 patients with epidemic influenza (Throat washings from these patients were passed serially in ferrets)

Epidemic	Num- ber of cases	Acute- phase blood		Convalescent phase blood		Increase in titer		
		Day mean	Titer mean	Day mean	Titer mean	Num- ber of cases with in- crease	Mean in- crease in titer	Per cent of cases with in- crease
1	6	2	1.9	60	1.61	6	25 x	100
2	24	3	1.14	27	1.130	23	18 x	96
3	16	1	1.14	27	1.194	15	38 x	94
4	14	4	1.69	26	1.424	12	25 x	86
Cases yielding virus	60	3	1.26	30	1.209	56	26 x	93
	27	3	1.16	30	1.196	27	28 x	100

x represents the arithmetical increase in the titer of the convalescent serum as compared with the titer of the acute serum

sera were used, the 1939 strains seemed less distantly related to PR8 than was the case with rabbit antisera, but remained very different from WS. Antisera against both strains, 149 and 188, failed to neutralize significant amounts of the swine virus.

Neutralization studies In the group of 65 patients with clinical epidemic influenza, acute and convalescent blood specimens have been tested for their capacity to neutralize the PR8 strain of epidemic influenza virus in 60 cases. The combined results of these tests are shown in Table VIII. The mean time after onset at which the acute-phase blood specimens were obtained was 3 days. The earliest specimen was taken on the 1st day and the latest on the 8th day. Ten of the so-called acute-phase blood specimens were actually preinfection specimens which were obtained 9 months before infection. All of these 10 sera came from patients in Epidemic 3. The mean neutralizing antibody titer of the 60 acute-phase sera was 1.26. The lowest titer was zero and the highest 1.360. The convalescent blood sera were obtained, on the average, 30 days after the onset of the disease. The shortest interval after onset was 22 days and the longest 66. The mean neutralizing antibody titer of the 60 convalescent

sera was 1.209. The lowest was 1.6, and the highest 1.444. The average increase in titer of the convalescent serum, as compared with the acute serum taken from the same patient, was 26 times. The lowest increase in titer was 4 times, the highest 160 times. Fifty-six of the 60 patients whose acute and convalescent sera were studied showed an increase in neutralizing titer of 4 times or more during convalescence. This represents 93 per cent of the cases studied. In Table VIII the results of neutralization tests on the acute and convalescent blood specimens from 27 of the 29 patients whose throat washings yielded influenza virus are shown separately. In the 2 remaining cases comparative neutralization tests were not done. The similarity between the results in the group which yielded virus and the total group is quite obvious.

In Epidemic 4, 20 cases were selected for weekly bleedings. The neutralization titer of each of these 80 blood specimens has been determined, and the results are shown in Figure 2. The day on which the blood specimens were taken after the clinical onset of the disease has been plotted against the logarithm of the dilution of serum which was capable of neutralizing 3,000 fifty per cent mortality doses of virus. The incremental rise in titer from 4 times to 256 times is graphically shown on the same scale.

The general trend of the rise in neutralizing antibodies following clinical epidemic influenza was similar in almost all of the 20 cases in this series. It appeared that the maximum neutralizing titer in these cases was achieved between the 10th and 14th day after onset. It is to be noted that approximately 50 per cent of the increase in antibody titer had been accomplished by the 7th or 8th day after onset. This fact is of considerable significance in interpreting the neutralizing antibody titer of sera taken late in the acute phase of the disease. As an example, it is possible that when a comparison is made between an acute-phase serum taken on the 7th day of the disease and a convalescent serum taken on the 20th day of the disease, an increase in neutralizing antibody titer of less than 4 times may be observed. Under these circumstances and if only an increase in titer of 4 times or more is considered to be significant, it will be concluded that no rise in neutralizing antibodies has taken place.

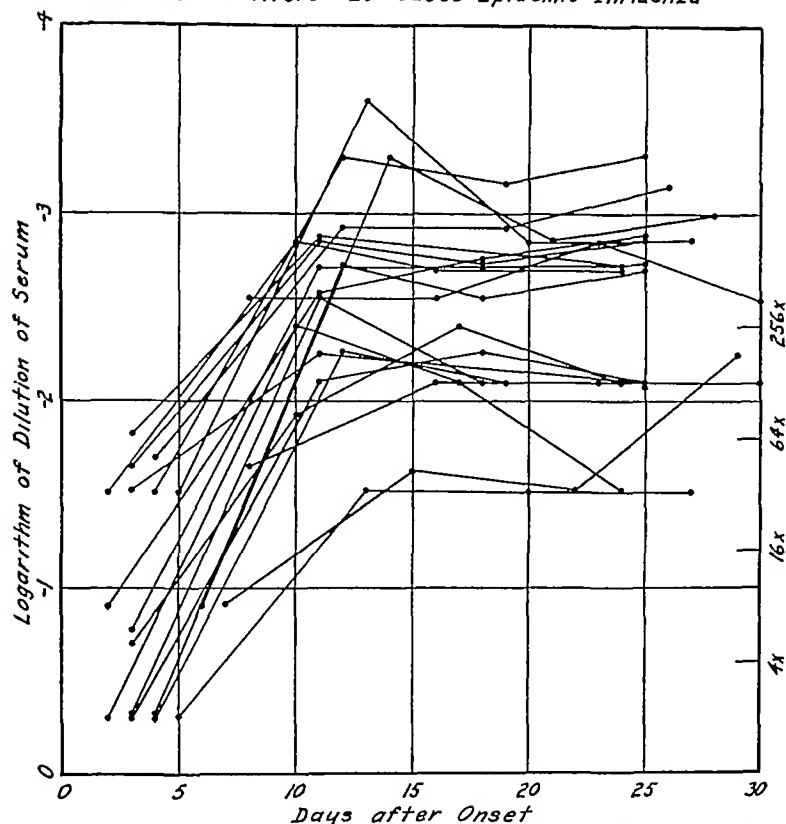
Neutralization Titers - 20 Cases Epidemic Influenza

FIG. 2. NEUTRALIZING ANTIBODY TITERS AT VARIOUS PERIODS AFTER THE ONSET IN 20 CASES OF EPIDEMIC INFLUENZA

Acute and convalescent blood sera have been studied from a total of 146 cases clinically diagnosed as epidemic influenza during 1939. These include the 60 cases already presented in Table VIII. The results of comparative neutralization tests on the 2 sera from each of these cases are presented in Table IX. The results of complement fixation tests on these sera will be reported separately by Eaton and Rickard (18). One hundred and fifteen or 79 per cent of the 146 cases showed an increase in neutralizing antibody titer

of 4 times or more during convalescence. Thirty-one cases failed to show a significant increase in neutralizing antibody titer during convalescence, but it should be pointed out that from the 22 patients in Epidemic 4 who failed to show an increase during convalescence the acute-phase blood was taken on the average, 8 days after the onset of the disease. In the case of the 8 patients in Epidemic 3 who failed to show a significant increase in neutralizing antibodies during convalescence, it may be of significance that the

TABLE IX

Results of comparative neutralization tests with epidemic influenza virus on two blood specimens from each of 146 patients with epidemic influenza

Epi- demic	Num- ber of cases	Acute phase blood	Conva- lescent phase blood	Increase in titer		
		Mean titer	Mean titer	Number of cases with increase	Increase in mean titer	Per cent of cases with increase
1	6	1.9	1.61	6	25 x	100
2	24	1.14	1.130	23	18 x	96
3	49	1.13	1.193	41	26 x	84
4	67	1.41*	1.262	45	26 x	67
	146	1.22	1.209	115	21 x	79

x represents the arithmetical increase in the titer of the convalescent serum as compared with the titer of the acute serum

* Twenty-five of the 67 acute-phase blood specimens have been excluded in the calculation of this titer since they were collected on the 8th day after onset or later

convalescent sera were compared with preinfection sera which were obtained from these persons, on the average, 9 months before the onset of the disease. The results of these neutralization tests are presented comprehensively in Figure 3, in which the relative accumulated frequencies of the different titer groups have been plotted against the observed titers. The two lines which have been drawn between the respective points for the acute and convalescent sera illustrate graphically

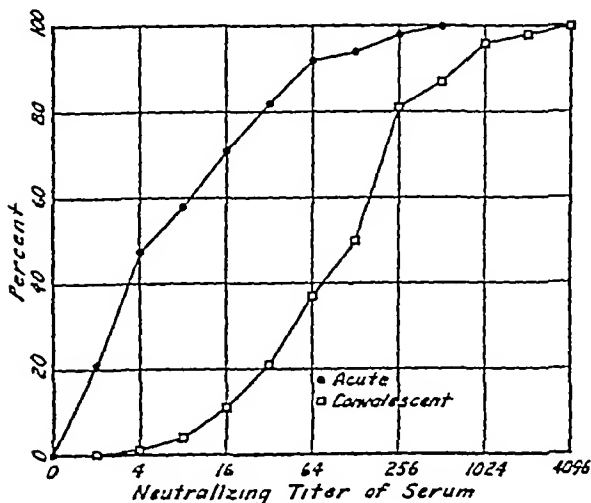


FIG. 3. RELATIVE ACCUMULATED FREQUENCIES OF NEUTRALIZING ANTIBODY TITERS OF ACUTE AND CONVALESCENT SERA FROM 146 CASES OF EPIDEMIC INFLUENZA

Twenty-five acute sera taken on the 8th day after onset or later have been excluded.

the distribution of neutralizing antibody titers in these sera. The area between the lines gives a graphic indication of the increase in titer which occurred during convalescence.

Contacts. Each of 33 persons who had contact with cases of epidemic influenza in the course of the four epidemics contributed two blood specimens, one during or before the epidemic and one after. Neutralization tests have been made with these sera, the results of which are presented in Table X. Complement fixation studies on these

TABLE X

Results of comparative neutralization tests with epidemic influenza virus on two blood specimens from each of 33 persons who had contact with cases of epidemic influenza

Group	Num- ber of cases	Intra- epi- demic blood	Post- epi- demic blood	Increase in titer		
		Mean titer	Mean titer	Num- ber of cases with in- crease	In- crease in titer mean	Per cent of cases with in- crease
Upper respira- tory illness	8	1.17	1.125	8	8 x	100
No illness	25	1.26	1.78	7	11 x	28
	33	1.23	1.81	15	10 x	45

x represents the arithmetical increase in the titer of the convalescent serum as compared with the titer of the acute serum

sera will be reported separately by Eaton and Rickard (18). Eight persons in the contact group suffered from mild and transient upper respiratory infections after the beginning of the epidemics, but in no instance were the symptoms considered to be similar to those seen in the cases diagnosed as epidemic influenza. Each of these 8 patients, however, showed a significant increase in neutralizing antibody titer in the second blood specimen. Twenty-five of the contact individuals had no history of respiratory illness during or immediately after the epidemic period. Nonetheless, 7, or 28 per cent, of this group also showed a significant increase in neutralizing antibody titer in the second blood specimen. In the contact group as a whole, 45 per cent of the 33 individuals showed a significant increase in neutralizing antibody titer in the second blood specimen. It seems reasonable to interpret these results as additional evidence for the possibility that subclinical infec-

tion with the virus of epidemic influenza can lead to an increase in antibodies equally as significant as that encountered in frank clinical cases. A comprehensive analysis of the results of the neutralization tests on the entire contact group is presented in Figure 4, in which the relative ac-

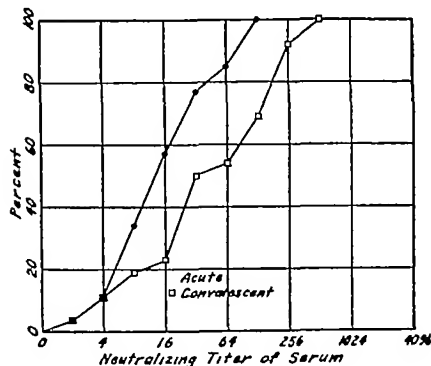


FIG. 4. RELATIVE ACCUMULATED FREQUENCIES OF NEUTRALIZING ANTIBODY TITERS OF INTRAEPIDEMIC AND POSTEPIDEMIC SERA FROM 33 INFLUENZA CONTACTS

cumulated frequencies of the various titer groups have been plotted against the observed titers. Although a significant increase in titer occurred in only 15 of the 33 cases this change was sufficient to displace considerably the line representing the convalescent titers.

Noninfluenzal infections. Eighty three cases of acute noninfluenzal infections of the respiratory tract have been studied in order to determine the relative levels of neutralizing antibodies against epidemic influenza virus in their acute- and convalescent phase sera. The results of these comparative neutralization tests are presented in Table XI. Two blood specimens from each of 56 patients with common colds were studied. A significant increase in neutralizing antibody titer was noted in only 1 case or 2 per cent. This individual was present in the community during Epidemic 3 and could have had contact with cases of epidemic influenza. Two blood specimens from each of 19 patients with sporadic grippé were studied and in no instance did a significant increase in neutralizing antibody titer occur during

TABLE XI

Results of comparative neutralization tests with epidemic influenza virus on two blood specimens from each of 83 patients with acute noninfluenzal respiratory infections

Clinical diagnosis	Number of cases	Acute-phase blood	Convalescent-phase blood	Increase in titer	
		Mean titer	Mean titer	Number of cases with increase	Per cent of cases with increase
Common cold	56	1.28	1.32	1	2
Sporadic grippé	19	1.35	1.36	0	0
Atypical pneumonia	5	1.6	1.6	0	0
Pneumococcus pneumonia	4	1.3	1.4	0	0
	83	1.27	1.30	1	1

convalescence. Two blood specimens from each of 5 cases with so-called atypical pneumonia and 4 cases of pneumococcus pneumonia were also studied and none showed a significant increase in neutralizing antibody titer during convalescence. One case among the total of 83 cases showed a rise in antibodies against epidemic influenza virus during convalescence, but it seems likely that, in this single instance at least, the increase in antibody titer could reasonably be explained by the possibility of contact with patients suffering from epidemic influenza. In Figure 5 are shown the results of a comprehensive

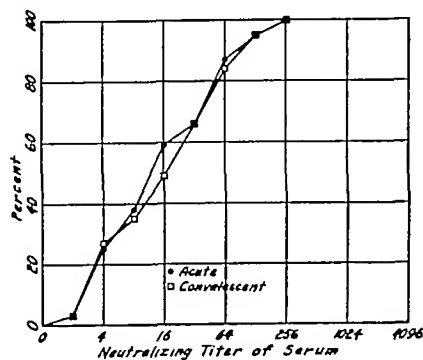


FIG. 5. RELATIVE ACCUMULATED FREQUENCIES OF NEUTRALIZING ANTIBODY TITERS OF ACUTE AND CONVALESCENT SERA FROM 83 CASES OF NONINFLUENZAL RESPIRATORY DISEASE

analysis of these cases. The relative accumulated frequencies of various titer groups have been plotted against the actual titers. The two lines representing the accumulated results for the acute and convalescent sera respectively have an almost identical position and illustrate graphically the fact that no significant change in titer occurs after these diseases.

In Figure 6 the accumulated frequency data for the acute-phase sera from 121 cases of epidemic influenza and similar data for the acute-phase sera from 83 cases of noninfluenzal respiratory disease are compared. These data illustrate

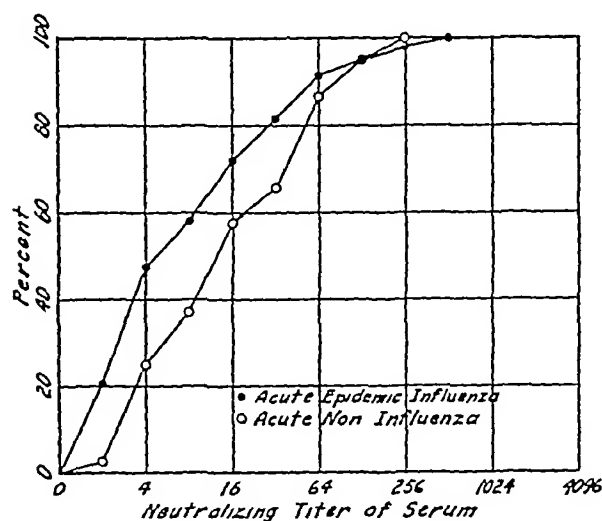


FIG. 6. RELATIVE ACCUMULATED FREQUENCIES OF NEUTRALIZING ANTIBODY TITERS OF ACUTE SERA FROM 121 CASES OF EPIDEMIC INFLUENZA AND 83 CASES OF NONINFLUENZAL RESPIRATORY DISEASE.

graphically the finding that the acute-phase neutralizing antibody titer was somewhat lower in epidemic influenza than in the noninfluenzal cases, but it does not seem likely that this difference is great enough to be significant.

DISCUSSION

The reports of previous investigators (1-12) have adequately proved that epidemic influenza is a definite disease entity with an established virus etiology. Unfortunately, there are no known pathognomonic signs or symptoms which permit an accurate clinical diagnosis. Although it has been suggested by Stuart-Harris *et al.* (12) that in the presence of an epidemic of respiratory dis-

ease a certain symptom complex may serve to differentiate influenza from similar but etiologically different diseases, the California epidemic studied by Francis (19) makes this possibility seem unlikely. In the four epidemics which have been studied during 1939 the clinical diagnosis was difficult, although the epidemic nature of the disease was obvious because of the close similarity of the clinical syndromes to those which had been observed in sporadic cases prior to the epidemic. In these latter cases influenza virus was not found in the throat washings, and no increase in neutralizing antibody titer was demonstrable during convalescence. It seems important to emphasize that, although a good correlation has been established in these four epidemics between the clinical diagnosis and the laboratory diagnosis of the disease, the clinical diagnosis was based largely upon the fact that epidemics had occurred and not upon any characteristic symptom complex.

The four epidemics have been characterized by relatively mild clinical disease which was almost uncomplicated. It may be that the mildness of the human disease was paralleled by a relative avirulence of the virus for ferrets, a possibility which has been suggested by Andrewes, Laidlaw, and Smith (3). In these epidemics considerable difficulty was encountered in obtaining evidence that the virus was present in the throat washings, even though serial passage in ferrets was carried out. Frequently, the first ferret inoculated failed to show a reaction which was sufficiently definite to indicate the presence of influenza virus. Even with prolonged serial passage of known strains of virus isolated this year, the responses were inconstant and 38 per cent of the passage ferrets failed to develop significant fever or symptoms. Additional evidence for the relative avirulence of the 1939 strains lies in the fact that they were not easily adapted to mice and were even less readily maintained in tissue culture. Despite this apparent avirulence for susceptible experimental animals, these strains of epidemic influenza virus seem to have been equally as good antigens as the more virulent strains previously isolated. Both experimental antisera against these strains and convalescent serum from patients with the disease possessed neutralizing antibody titers comparable to those produced by the most virulent strains. Not only have the 1939 strains been less virulent

for experimental animals than those isolated in previous years but their isolation from the throat washings of serologically proven cases has been less frequent than in previous years. Virus has been obtained from throat washings in only 45 per cent of instances whereas it was obtained in more than 70 per cent of cases in 1937 (11, 12).

The study of the neutralizing antibody titers of acute and convalescent blood specimens from each of 83 cases of noninfluenza respiratory infection conclusively demonstrated that no rise in titer against epidemic influenza virus occurred during convalescence from these diseases. In contrast to this the increase in neutralizing antibody titer noted during convalescence in the large majority of the 146 cases of epidemic influenza was striking. The results of the neutralization tests on the sera obtained at weekly intervals from cases in Epidemic 4 indicate that a rapid increase in neutralizing antibody titer occurred after the onset of the disease.

The contact group presents certain interesting findings. Among the individuals who had contact with cases of epidemic influenza but gave no history of any respiratory infection thereafter 28 per cent showed an increase in neutralizing antibodies against the virus in their second blood specimens. This seems good evidence that subclinical infection by epidemic influenza virus had occurred. Somewhat similar evidence has been presented by Stuart-Harris *et al.* (12), and an identical conclusion was reached by Francis *et al.* (9) on the basis of the antibody titers found in the serum of contacts after an epidemic. The individuals in the contact group who did develop mild upper respiratory infections require special consideration since all of these individuals showed an increase in neutralizing antibodies in their second blood specimens. In none of these cases were the symptoms consistent with a diagnosis of influenza and in most instances "common cold" seemed the most suitable classification. The precontact levels of neutralizing antibodies in these two groups were almost identical and do not offer any explanation for the fact that, following contact illnesses occurred in the first group but not in the second.

Two possibilities seem likely. (a) The mild clinical symptoms were unrelated to a subclinical infection by epidemic influenza virus and, therefore, to the increase in neutralizing antibodies, or

(b) The virus produced in these individuals, an unusually mild and aberrant form of the disease which was followed by an increase in antibodies. Both possibilities have some support from the fact that influenza virus previously has been isolated and an antibody rise demonstrated by Francis *et al.* (11) in (a) a contact who did not develop any symptoms and (b) a contact who had minimal symptoms for 24 hours.

The certain diagnosis of epidemic influenza probably cannot yet be made upon either clinical or epidemiological grounds. Neither can the diagnosis be made solely by serological laboratory tests, since persons who have contact with cases but themselves remain symptom-free, may develop an increase in antibodies equal to that shown by frank cases. Therefore a clinical history becomes an essential supplement of the laboratory studies. A certain diagnosis of epidemic influenza still seems to require three types of evidence: (a) clinical history, (b) isolation of virus, and (c) increase in antibody titer against the virus. It seems reasonable to suggest that a definite distinction be made between the disease epidemic influenza and infection by epidemic influenza virus. The former is characterized by a variable symptom-complex: the presence of virus in the nasopharynx, and an increase in antibody titer during convalescence. The latter may be entirely symptomless but nonetheless is followed by an increase in antibody titer.

SUMMARY

1 Epidemic influenza virus was isolated from cases of influenza in four epidemics during 1939. The virus was not isolated from various cases of acute respiratory disease which occurred prior to the epidemics.

2 The strains of virus isolated produced inconstant reactions in ferrets and the experimental disease was typically mild. The strains were not easily adapted in mice.

3 Antigenically these strains were more nearly related to the PR8 strain than to the WS strain but were different from both.

4 The neutralizing antibody titer increased rapidly after the 5th day of disease and reached maximum levels between the 10th and 14th days.

5 Contacts developed a significant increase in

neutralizing antibody titer without manifesting any symptoms

6 No increase in neutralizing antibody titer occurred during convalescence from four different noninfluenzal respiratory infections

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STUDIES OF RHEUMATIC DISEASE III FAMILIAL ASSOCIATION AND AGGREGATION IN RHEUMATIC DISEASE

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At the present time the available evidence is insufficient to establish definitely the etiology of rheumatic disease although promising work has been done and is in progress. It has long been recognized that there is a tendency for the disease to occur more frequently in some families than in others. In a previous study (1) based upon the records of the families of patients admitted to the Cardiac Clinic of the Harriet Lane Home careful statistical analysis suggested that hereditary constitution might be a factor in causing this aggregation. The evidence presented however did not exclude the possibility that the familial aggregation might be due solely or principally to common exposure either to a microbic cause and/or to any environmental factor such as nutritional deficiency which favored development of the disease.

If the disease were due solely to heredity it would be expected that it would manifest itself in the various members of the family in such a way that its first acute manifestations (onsets) would be scattered along the time scale of family experience in a random fashion or affected solely by the age of its members. On the other hand, if in addition to hereditary predisposition, an environmental factor or factors were concerned it would be expected that during certain periods of time in the family experience the risk of attack would vary according to the degree with which the environmental factor was acting. Thus we would expect a certain amount of grouping of the onsets of new cases occurring in the family and after one member had developed acute manifestations of the disease there would be an increased incidence among his familial associates. This expectation would be justified regardless of the environmental factor involved whether it be an infective agent and/or some other common environ-

mental factor necessary for the development of the disease. In other words, a tendency toward the grouping or aggregation of new cases within the family would appear at certain times, as in the case of infective disease such as diphtheria scarlet fever tuberculosis and in nutritional deficiencies like pellagra.

To elucidate this relationship the records of the group of families previously used in the study of the hereditary factors have been analyzed to determine the relationship of the first appearance of rheumatic manifestations¹ (onsets) in the various members of these families along a time axis.

MATERIAL AND METHODS

The data upon which this report is based have been extracted from the medical records of 96 consecutive admissions of white children to the Cardiac Clinic of the Harriet Lane Home because they were suffering from rheumatic disease, and from the epidemiological record of this disease in their parents and siblings. A manifestation of rheumatic disease was defined as chorea, rheumatic fever or rheumatic heart disease, and this definition is the same as that used in the two preceding articles (1, 2). Detailed information for each individual in 95 of these families is available with respect to the date of birth, date of last observation, and the date of onset, duration, and type of each manifestation of rheumatic disease. The data relative to the rheumatic manifestations have, in most instances, been confirmed by hospital records. The relationship between the acute manifestations of rheumatic disease in the various members of each family and the age of each individual when exposed to these attacks can be ascertained from these reports. As an aid in this analysis, family graphs were drawn for each family showing the intrafamilial relationship of all attacks of rheumatic disease. A sample graph is shown in Figure 1.

¹ This analysis deals only with the time relationship of primary onsets. Second and subsequent attacks in any member have been considered as recurrences.

² One family in which the records are incomplete has been excluded from the study.

HARRIET LANE HOME RHEUMATIC FEVER STUDY

Family No: 21 Name N Race: White

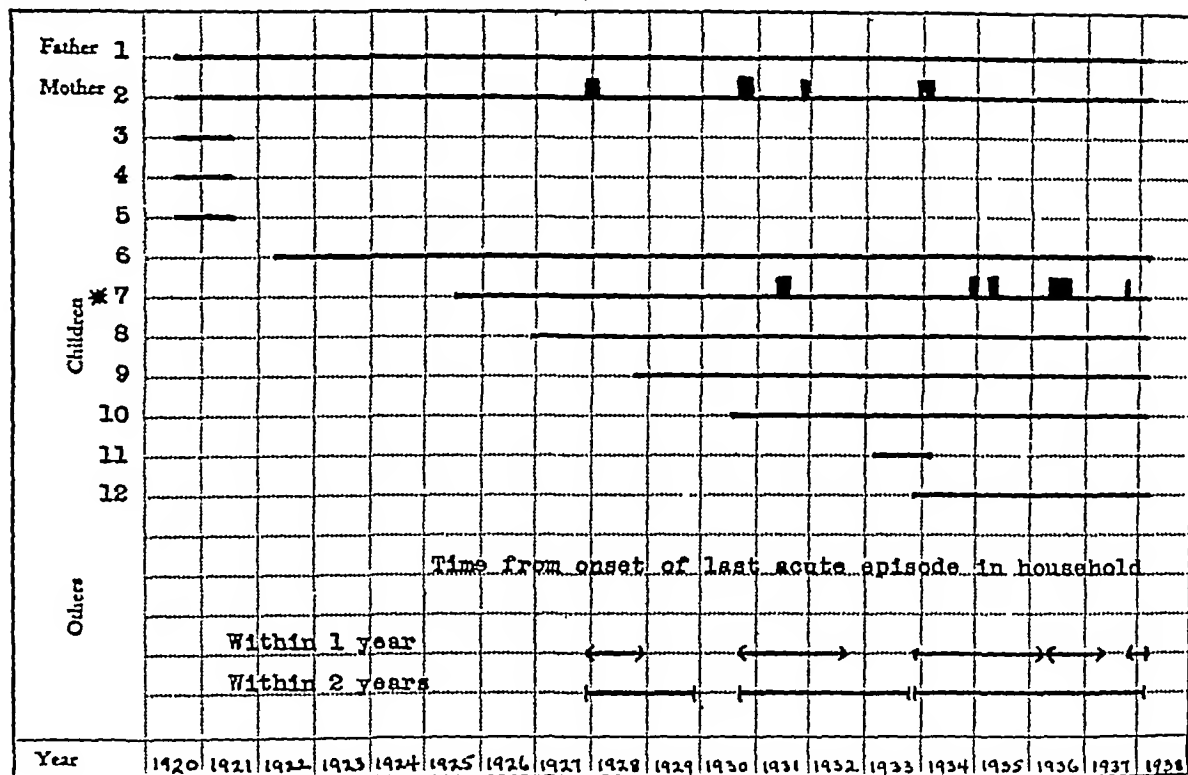


FIG 1 FAMILY GRAPH

Solid line indicates life-experience in the household. Acute episodes are indicated by solid blocks on top of line.

In order to hold the factor of heredity constant, as far as possible, the families were divided according to whether or not their parents had a history of rheumatic disease. There were 43 families in the group in which one or both parents were rheumatic, and 52 families in which neither parent had a rheumatic history. The children of these families have been studied to determine if an increased risk of developing the disease was associated with the occurrence of an acute episode of the disease in any other member of the household (adult or child).

It should be noted that the 95 families under study were selected because at least one member (a child) was admitted to the clinic suffering from rheumatic disease. The children who are responsible for the inclusion of their families in the study are known as "index cases" to distinguish them from their siblings who enter the study because of their familial relationship. The index case is not necessarily the first case to occur in the family, but it is the first to come to the clinic suffering from the disease. Because index cases are the means through which the families are selected and, by definition, must have had an attack of rheumatic disease, they have been excluded from the tabulations in order to avoid bias and attention has been confined to the occurrence of the dis-

ease in their siblings who are unselected, except with regard to their familial relationship.

The final composition of the group studied was

	Number of families	Number of siblings of index cases
Rheumatic disease in parents	43	156
No rheumatic disease in parents	52	191

The life-experience of these siblings, in each of the above groups, has been divided into that which preceded familial association³ with another member of the household (adult or child, including index cases) suffering from an acute manifestation (primary or recurrence) and that which followed such association. By this procedure it is possible to compare the incidence of new cases in the children during these two periods.

The occurrence of rheumatic disease in the children of these families could be expressed as the percentage who

³ The word association is preferred because the terms "exposure" or "contact" would imply that the effective environmental factor is opportunity for transmission of an infective agent. For present purposes it is desirable to avoid this assumption.

had the onset of their first attack before, as compared to after association with an acute episode, but such a statement is not fully satisfactory because it fails to take into consideration the time during which each was at risk during these periods and the possible differences in age susceptibility. For this reason the experience of these children has been recorded in the terms commonly applied in the general population to express incidence of any disease, namely the age-specific annual rates of morbidity.

In order to state the observations on this group in these terms it is necessary to reduce the prior and subsequent experience to a comparable base. This can be done by expressing the experience of these children in person-years of life before and after the date of the first intra-familial association with an acute episode of the disease. By this method consideration can be given not only to the number of children in the family group but also the length of time and the various ages during which each child was under observation.

If the purpose is to calculate morbidity prior to familial association with an acute attack, the age at which observation begins is at birth because all of these children entered their respective families at birth. The age at which observation ends is dependent upon the occurrence of the first of four possible events (1) death, (2) familial association with an acute attack in another member of the family (3) onset of rheumatic disease, and (4) termination of observation without association. If the purpose is to show morbidity subsequent to association the observation begins with the age attained when first associated with an acute attack and ends with either (1) death, (2) onset of rheumatic disease, or (3) termination of observation. The actual procedure necessary to convert the observations into person-years of experience is that described by Frost (3) and Stewart, Gass, Gauld and Puffer (4) in their studies on tuberculosis.

Incidence of rheumatic disease in the children of the families of rheumatic index cases prior and subsequent to exposure to acute rheumatic manifestations in any member of the household

The experience of the children in the study group expressed in person years along with the cases of rheumatic disease and the annual morbidity rates are shown in Table I. In this table the children are grouped according to the history of rheumatic disease in their parents and the experience divided into that which preceded and that which followed first association with an acute episode.

In calculating the total incidence of rheumatic disease for these two periods, only the experience of the children after their second birthday has been used. This has been done because the oc-

TABLE I

Life-experience cases of rheumatic disease and annual attack rates among the siblings of 95 rheumatic index cases prior and subsequent to first association with an acute manifestation of the disease and according to the history of rheumatic disease in the parents

Age	Prior to association with an acute episode			Subsequent to association with an acute episode		
	Person-years	Cases	Rate per 100	Person-years	Cases	Rate per 100
NO PARENTAL HISTORY OF RHEUMATIC DISEASE						
Under 2	338.5			20.50		
2-4	425.5			62.50	2	3.20
5-14	706.5	10	1.41	336.00	9	2.68
15+	89.5	2	2.23	121.75	2	1.64
Total over 2 years	1221.5	12	0.98	520.25	13	2.50
PARENTAL HISTORY OF RHEUMATIC DISEASE						
Under 2	243.0			48.50		
2-4	257.0	4	1.55	128.25	3	2.34
5-14	305.0	6	1.96	397.50	18	4.53
15+	17.0			99.00	4	4.04
Total over 2 years	579.0	10	1.73	624.75	25	4.00

currence of rheumatic manifestations under two years of age is rare, and no cases occurred during this age period in these families.⁴

When the annual rates for all ages over two years are compared before and after association it is found that the incidence in both groups of children is more than doubled in the period following association with an acute episode in the household. In these children whose parents gave no history of rheumatic disease, the rate increased from 0.98 to 2.50 per hundred per year while in those whose parents had a history of rheumatic disease the increase was from 1.73 to 4.00. Although based upon a comparatively small experience these differences are sufficiently large to have statistical significance, the probability of a difference of this size occurring by chance in this group is only two in one hundred trials.

It should be further noted that although the experience in each age group is not large enough to give statistical significance to the differences with

⁴ The effect of this deduction from the total life experience has been to minimize the differences found between the incidence in the two periods.

one exception the age-specific rates are consistently higher after familial association with the acute disease

From the data in Table I it is also possible to compare the incidence in children of rheumatic and non-rheumatic parents. This comparison may be expressed in a ratio as follows

Ratio of incidence

$$\frac{\text{Children of rheumatic parents}}{\text{Children of non rheumatic parents}} \times 100$$

Prior to association with an acute episode $\frac{176}{100}$

Subsequent to association with an acute episode $\frac{160}{100}$

It is interesting to note that the incidence is 60 to 80 per cent higher in the children of rheumatic parents than in the children of non-rheumatic parents, and that this ratio is not materially altered by association with an acute manifestation of the disease

The time relationship between association and the onset of new cases occurring subsequently

The analysis up to this point has dealt with the total incidence of new cases of rheumatic disease among siblings of index cases which preceded and followed the first familial association with an acute episode. This fails to take into consideration the fact that a child might be associated with more than one acute episode in other members of the family before showing clinical evidence of having contracted it. If the occurrence of an acute episode in one member of the family is an indication that an environmental factor or factors, which have etiological significance, are operating at this particular time, then it would be expected that during the time interval closely following this event the risk of attack would be increased for other members in the same family. This in turn would be reflected in an increased case rate during a proximal subsequent period as compared with later periods.

In Table II is shown the distribution of 38 cases which occurred among the siblings of index cases according to interval elapsing between the time of association with the last prior acute episode and the time of onset.

TABLE II

Time interval between last prior association with an acute episode in another member of the family and the onset of rheumatic disease in 38 cases

Interval	Number of cases
Less than 1 year	15
1-2 years	8
2-3 years	5
3-4 years	1
4-5 years	5
5-9 years	2
Over 10 years	2
Total	38

It will be noted that 15 of the 38 cases had their onset within one year, and 23 (15 + 8) within two years of their last prior association with an acute episode in another member of the family. At first glance the distribution suggests that the risk is greatest among these children within a year or two following association with an acute attack. This tabulation, however, does not show the number of cases in proportion to the number of persons still under observation in the stated intervals and, accordingly, does not indicate the relative risk in these periods.

TABLE III

Showing the incidence of rheumatic disease subsequent to first association with an acute manifestation according to its proximity in time to acute attacks in other members of the family

Time from last association with an acute episode	Person years experience	Cases onset during period	Rate per 100 person years
PARENTS—NO HISTORY OF RHEUMATIC DISEASE			
Less than 1 year	257.50	7	2.7
1 to 2 years	120.75	4	3.3
More than 2 years	142.00	2	1.4
PARENTS—HISTORY OF RHEUMATIC DISEASE			
Less than 1 year	312.25	8	2.5
1 to 2 years	130.50	4	3.1
More than 2 years	182.00	13	7.1

Using the onset of each separate acute episode as a focal point, it has been possible to divide the total experience of each child, subsequent to first association, according to its proximity to the onset of the last acute attack in another member of the family. This division was made in three time

bands (1) the experience which fell within one year following association with an acute episode (2) that which was more than one year and less than two years following association, and (3) that which was more than two years following association.*

After the total experience following first association of each child was divided in this way that of the group as a whole was obtained by adding the individual experiences together. Rates can be calculated for each interval by dividing the cases

* In making this division, the procedure was to calculate for each individual the amount of experience following first association which was within one year and that which was within two years of association with an acute episode in the household. The detail for calculations on individual 6 Family 21 (shown graphically in Figure 1) was

Beginning of association		End of period		Episodes with which associated		Number of months in period
Date	Age	Date	Age	Date of onset	Individual attacked	

A. EXPERIENCE WITHIN ONE YEAR OF BEGINNING OF ASSOCIATION WITH AN ACUTE EPISODE

December 1927	5	December 1929	5	December 1927	No. 2	12
September 1930	8	November 1932	10	September 1930	No. 2	
				June, 1931	No. 7	26
				November 1931	No. 2	
				December 1932	No. 2	
				December 1934	No. 7	
				April, 1935	No. 7	
May, 1934	14	May 1937	18	May, 1935	No. 7	20
October 1937	15	February 1938	15	October 1937	No. 7	13
						5
						54

B. EXPERIENCE WITHIN TWO YEARS OF BEGINNING OF ASSOCIATION WITH AN ACUTE EPISODE

December 1927	5	December 1929	7	December 1927	No. 2	24
September 1930	8	November 1932	11	September 1930	No. 2	
				June, 1931	No. 7	38
				November 1931	No. 2	
				December 1932	No. 2	
				December 1934	No. 7	
				April, 1935	No. 7	
December, 1933	11	February 1938	15	May, 1935	No. 7	51
				October 1937	No. 7	113

C. TOTAL EXPERIENCE SUBSEQUENT TO FIRST ASSOCIATION WITH AN ACUTE EPISODE

December, 1927	5	February 1938	15	No. 2 and No. 7	122
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Final allocation of experience:

Experiences within one year of association (A) $\frac{54}{12} = 7$ person-years

Experiences more than one and less than two years following association (B-A)

$$\frac{113-54}{12} = 2\frac{1}{2} \text{ person-years}$$

Experiences more than two years following association (C-B) $\frac{122-113}{12} = \frac{10}{12}$ person-years

having their onset within it by the corresponding total person years experience. These rates express the annual incidence of the disease in those at risk according to the proximity to all acute episodes with which the children were associated.

Although the experience is small, it shows no tendency for new cases appearing in other members of the family to be grouped or aggregated closely about the time at which one member comes down with acute manifestations. Indeed if any inference at all is permissible it is the contrary one: that after the disease has occurred in one member of the family it may be a matter of years rather than of months before another member is attacked.

SUMMARY AND DISCUSSION

The children of 95 families in each of which one child entered the clinic because of some rheumatic manifestation, were studied with respect to the relationship of the occurrence of the disease among them to familial association with an acute episode of the disease in another member of the family. As far as possible hereditary factors were held constant throughout the analysis, and the index cases were excluded from the tabulations because of the bias which they introduced.

The analysis showed that the risk of contracting the disease among the siblings of the index cases was increased, following association with an acute episode in another member of the family to more than twice that which prevailed prior to this association. This suggests that there is an environmental factor which plays a role in the causation of this disease.

The children of rheumatic parents had higher attack rates than the children of non rheumatic parents both before and after their first familial association with an acute episode. The interpretation of this finding should be made with caution because the children who have parents with a rheumatic history are in most instances, also associated with what might be called the chronic quiescent phase of the disease in these parents. The higher incidence in these children could therefore be due either to an increased hereditary susceptibility or to long continued association with the disease in chronic form. Considered along with the findings of the previous article (1) the first would seem to be the more probable explanation.

tion, *i e*, that heredity plays a definite role in the etiology of the disease

The time relation between episodes in the family and the occurrence of subsequent cases in other members did not show a definite tendency for the incidence of subsequent attacks to be highest within short time intervals of an association with acute episodes. This finding would suggest that either long continued exposure to the cause (whether it be parasitic or non-parasitic) is necessary, or that the disease is slow in developing to the point where it becomes clinically manifest. In this respect, if it be due to an infection, it therefore resembles tuberculosis rather than an acute infection such as scarlet fever or diphtheria, and the results of exposure in any household should not be measured in weeks or months but in years.

These findings are consistent with the hypothesis that in the etiology of rheumatic disease there are both hereditary and environmental factors involved, and that the environmental factor to produce the disease must act over a long period of time, and/or the disease has a long period of sub-clinical development before becoming manifest. They are consistent with such an hypothesis, but do not prove it, because other explanations could fit the observed facts.

CONCLUSIONS

Careful observations over varying periods of time on 347 siblings of 95 children who were ad-

mitted to the clinic because of some rheumatic manifestations showed that the attack rate

1 Increased after association with an acute episode

2 Was higher among the children of rheumatic parents both before and after such association

3 Showed no tendency, following association with an acute episode, to be higher within a short proximal period as compared with a more remote later period

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URINARY EXCRETION OF THIAMIN IN CLINICAL CASES AND THE VALUE OF SUCH ANALYSES IN THE DIAGNOSIS OF THIAMIN DEFICIENCY¹

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An accurate and specific method for the determination of thiamin in urine based on the reaction between the vitamin and diazotized p-aminoacetophenone has been developed (1). Under suitable conditions, such determinations should give information regarding the state of saturation of an organism with this vitamin. Studies of the urinary excretion of thiamin in normal subjects before and after test doses, the factors influencing such excretion and the changes associated with experimental deficiency have been reported (2). The present communication deals with determinations of the urinary excretion of thiamin in 89 patients in the University Hospital, correlation of these values with dietary histories of these patients, and the evaluation of the possible association of thiamin deficiency with the clinical conditions encountered.

CONDITIONS AND METHODS

The thiamin content of the diet ingested by each patient during the month prior to the test was evaluated on the basis of repeatedly consistent details of the dietary history as obtained by more than one questioner. This was classified as deficient only when obviously so as suboptimal when the diet was low in protective foods but not grossly deficient and as adequate when estimations indicated an intake of two-thirds of a milligram (220 international units) or more.

Two consecutive 24-hour urine specimens were collected from each patient. Just prior to the beginning of the second sample and after the largest

meal of the day an aqueous solution of 5 mgm of thiamin was taken orally. Previous studies (2) indicate the advisability of using the oral route for the test dose and the necessity of giving it with a meal. The first sample was analyzed to give the 24-hour excretion value when the diet furnished the entire supply of the vitamin. As the patients ate the same diet on the 2 consecutive days, the value for the first sample was subtracted from the value obtained by analysis of the second sample in order to calculate the percentage of the test dose excreted in the 24 hours following its administration. The method of analysis has been described in detail elsewhere (1).

Since drastic reduction of the dietary thiamin of normal subjects resulted in a rapid decrease of urinary thiamin excretion (2) the thiamin content of the diets ingested on the days of the tests is estimated in the tables. When numerical values are given, calculations are based on the tables compiled by Williams and Spies (3). Unless otherwise indicated the tests were done before the fifth day of hospitalization. The absence of an appreciable increase in the urinary excretion during the first few days after resumption of a normal thiamin intake by a subject with experimental deficiency (2) demonstrates that the ingestion of an adequate diet by the deficient individual for a few days prior to the test does not vitiate the significance of subsequent values.

RESULTS

Standards. Standards for the interpretation of values obtained with chief attention directed to the minimal normal excretion of thiamin have been derived by correlating the urinary thiamin values for each subject with the adequacy of the dietary thiamin intake during the preceding month in a series of 24 normal controls (2), 22 hospital patients without clinical evidence of nutritional

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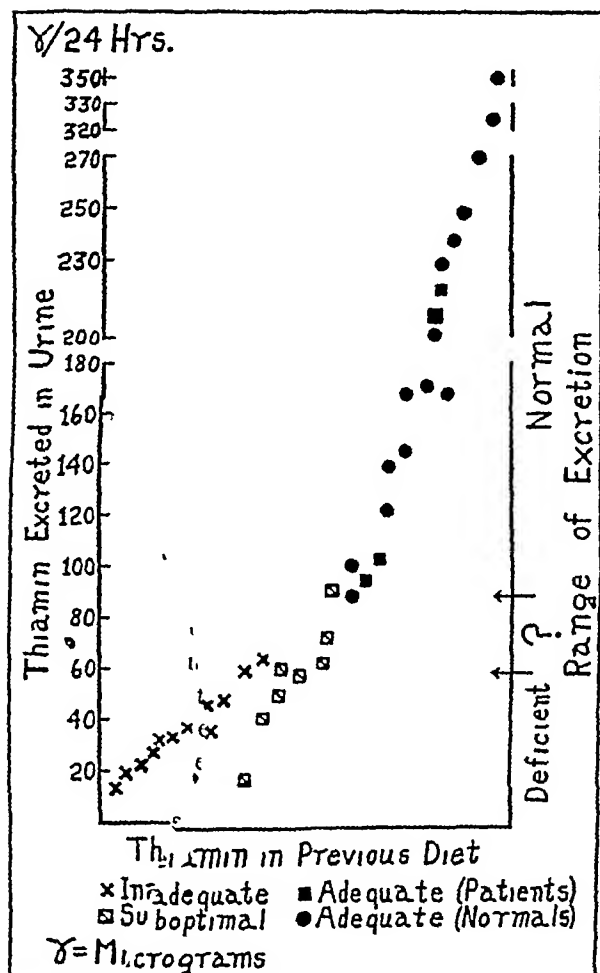


FIG. 1. CORRELATION IN ADULT MALES BETWEEN THE ADEQUACY OF THE PRECEDING DIETARY THIAMIN INTAKE AND THE 24-HOUR URINARY THIAMIN EXCRETION

deficiencies, and 24 patients with clinical evidence of deficiencies in one or more nutritional factors, including 3 patients with "alcoholic beriberi." All patients were excluded who had disorders which might lead to faulty absorption, storage, utilization or excretion of the vitamin.

Figure 1 presents the values of urinary thiamin excretion by male subjects during the time that the diet furnished the entire source of thiamin. Those whose preceding diets had been definitely inadequate excreted 66 micrograms or less per 24 hours, whereas all subjects who had previously been on adequate diets excreted 90 micrograms or more. With the female subjects (Figure 2) the division is not so sharp, but no subject who had taken an adequate thiamin intake excreted less

than 53 micrograms per 24 hours and only 1 excreted less than 60 micrograms. Only 1 female with a history of a definitely inadequate diet excreted more than 43 micrograms. Since no significant difference was seen between males and females in the per cent of the oral dose excreted, these data for both sexes are presented in Figure 3. All patients with a history of a preceding inadequate thiamin intake excreted less than 7 per cent of the oral dose, all 27 subjects whose diets had been adequate excreted about 8 per cent or more, and 22 of the 27 excreted 10 per cent or more.

It appears that if a male subject fails to excrete more than 90 micrograms of thiamin during a 24-hour period when he is ingesting an adequate diet, he may be suspected of having a significant reduction of the body stores of thiamin, and if he excretes less than 60 micrograms, such reduction is reasonably certain. Corresponding values for females place the lower limit of normal excretion at 60 micrograms, with values below 40 micrograms

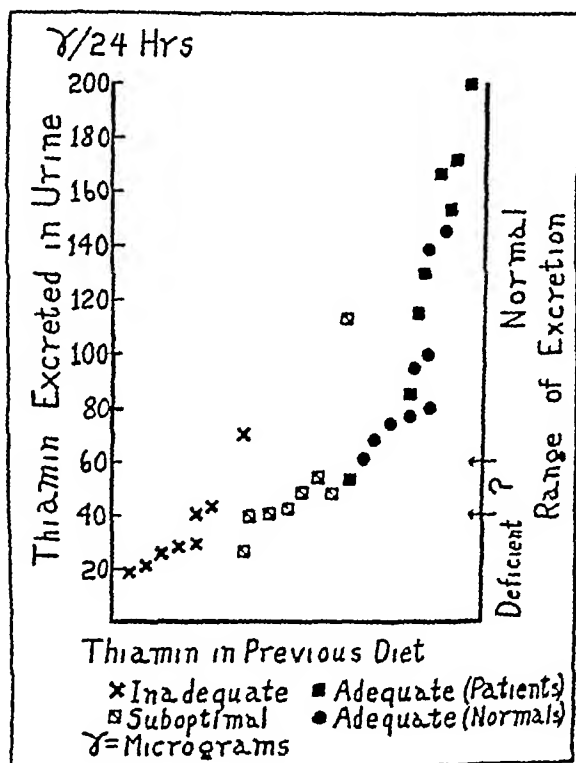


FIG. 2. CORRELATION IN ADULT FEMALES BETWEEN THE ADEQUACY OF THE PRECEDING DIETARY THIAMIN INTAKE AND THE 24-HOUR URINARY THIAMIN EXCRETION

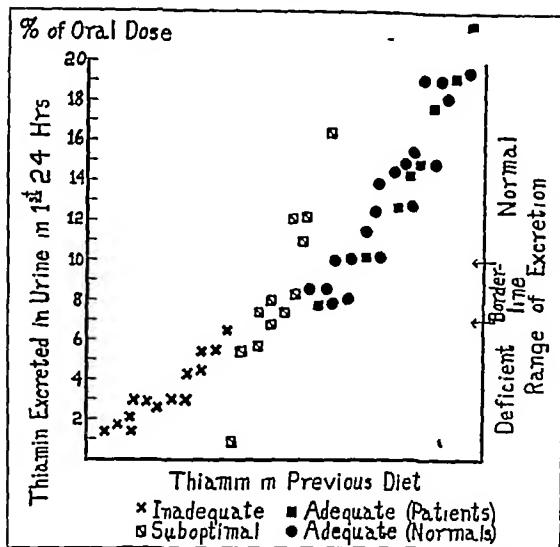


FIG. 3 CORRELATION IN ADULT MALES AND FEMALES BETWEEN THE ADEQUACY OF THE PRECEDING DIETARY THIAMIN INTAKE AND THE PER CENTAGE OF A 5 MG. TEST DOSE OF THIAMIN EXCRETED IN THE URINE DURING THE FIRST 24 HOURS AFTER IT IS TAKEN ORALLY WITH A MEAL.

The excretion values are corrected for the amount of thiamin from dietary sources excreted during the test period.

indicative of a fairly certain depletion. Excretion by a member of either sex of less than 7 per cent of a 5 mgm. oral test dose taken with a meal is also evidence of depletion of thiamin stores. It is pertinent to note that values for the normal excretions used in estimating these standards are obtained from subjects in whom no attempt at saturation with the vitamin has been made. These minimal standards for thiamin excretion are purposely conservative.

Patients with clinical thiamin deficiency. Three patients with peripheral polyneuritis clinically identical with that of beriberi (4) were studied. Case 1 in addition presented all the findings of 'beriberi heart disease' (5). Alcoholism was responsible for a grossly inadequate dietary intake in each case. In these patients the values for thiamin excretion, presented in Table I are much below those of the controls both before and after the oral dose. Each of these patients also ex-

TABLE I
Urinary excretion of thiamin by patients with clinical thiamin deficiency

Case number	Sex	Age	Clinical diagnosis	Thiamin content of previous diet	Dietary thiamin during test			Fraction of oral dose excreted in urine
					Before oral dose	After oral dose		
		years			micrograms per 24 hours	micrograms per 24 hours	micrograms per 24 hours	per cent
1	M	35	Peripheral polyneuritis and wet beriberi	Inadequate	360	13	120	2.1
			Three days after cessation of treatment		360	398	1000	12.0
2	M	61	Peripheral polyneuritis*	Inadequate	530	50		
			Two days after cessation of treatment		630	240		
3	M	64	Peripheral polyneuritis	Inadequate	870	19	223	4.3

* This patient also had clinical evidence of scurvy and a plasma ascorbic acid value of 0.17 mgm. per 100 cc.

creted definitely less thiamin in the urine after parenteral test doses than did normal subjects. All 3 patients showed excellent clinical improvement after treatment with crystalline thiamin, adequate diets, and other vitamin supplements as indicated by coincident deficiencies.

After treatment, Cases 1 and 2 returned to the diets on which the original tests were done and showed urinary excretions in the high normal range. The high urinary excretion after treatment, when no test dose was given, may be due, in part, to excess storage of the vitamin given therapeutically. Subsequent studies (2) have indicated that a longer interval is required for the urinary thiamin excretion of a normal subject to

return to its basal level after discontinuation of supplementary thiamin.

Patients with other nutritional deficiencies
Twenty-one patients who presented clinical evidence of single or multiple nutritional deficiencies as a major, and often the sole, cause of symptoms were studied. The results are presented in Table II. None of these cases presented unequivocal clinical evidence of thiamin deficiency. It should be noted that we have made the diagnosis of pellagra in patients who have manifestations other than the classical dermatitis, and that many foods with a fair to good thiamin content (3) are not highly pellagra-preventive (6). The clinical diagnosis in these cases was confirmed by observation of a significant response to therapy with appropriate vitamin supplements. All but 1 patient showed excretions before the test dose definitely lower than the values obtained on normals, and 9 of the 18 cases given the oral test dose excreted less than 7 per cent thereof. Cases 5 and 19 show apparently paradoxical values, with low excretion on the diet alone and unusually high values after the oral dose. The tests were repeated on Case 15 8 days after the cessation of treatment with a yeast and liver concentrate of high thiamin content. Values well in the normal range were obtained then.

Patients with organic heart disease
The fact that thiamin deficiency *per se* can cause serious cardiovascular disturbances (5, 7, 8) has led to consideration of the possibility that a concomitant but less evident deficiency may contribute to the clinical disability in organic heart disease (9, 10, 11). Restriction of the intake of the "protective foods" is not uncommon in chronic cardiac disease, either as a result of gastro-intestinal symptoms or as part of the therapeutic regimen. Table III presents the urinary thiamin excretion values of 7 patients who were hospitalized for treatment of cardiac decompensation. There was no evidence of impairment of renal function in these cases. Case 31, the only one to show excretions definitely in the normal range, was tested after recovery from decompensation. However, all 7 patients responded satisfactorily to the usual treatment with bed rest, digitalization and diuretics, on an adequate diet without vitamin supplements. Cases 26, 28, and 29 failed to show any additional

TABLE II

Urinary excretion of thiamin by patients with nutritional deficiency

Case number	Sex	Age	Clinical diagnosis	Thiamin content of previous diet	Dietary thiamin during test	Thiamin excreted in urine		Fraction of oral dose excreted in urine
						Before oral dose	After oral dose	
		years				micrograms per 24 hours	micrograms per 24 hours	per cent
4	F	40	Pellagra	Adequate	Good	167	882	14.3
5	F	19	Pellagra	Suboptimal	Fair	29	1308	25.6
6	F	33	Pellagra	Suboptimal	Fair	39	853	18.4
7	F	53	Pellagra	Suboptimal	Good	48	600	11.0
8	F	19	Pellagra, and migraine	Suboptimal	Low	42	414	7.4
9	F	38	Pellagra	Suboptimal	Fair	54		
10	F	18	Pellagra—"anorexia nervosa"	Inadequate	Fair	43	366	6.5
11	F	44	Pellagra and psychoneurosis*	Inadequate	Low	26		†
12	F	37	Pellagra and hyperemesis gravidarum	Inadequate	Good	28	300	5.4
13	F	34	Pellagra	Inadequate	Low	18	292	5.5
14	F	47	Pellagra	Inadequate	Fair	21	170	3.0
15	F	17	Pellagra and microcytic anemia	Inadequate	Good	40	270	4.6†
16	M	29	After treatment	Good	Good	208	1765	31.1†
17	M	24	Angular stomatitis	Suboptimal	Fair	62	668	12.1
18	M	61	Hypovitaminosis A*	Suboptimal	Low	52	660	11.0
19	M	57	Pellagra	Suboptimal	Good	92	450	7.4
20	M	81	Pellagra and bizarre gastritis	Inadequate	Fair	37	1235	25.0
21	M	61	Scurvy and pellagra*	Inadequate	Low	22		†
22	M	64	Pellagra and neoplasia of jaw	Inadequate	Good	32	180	3.0
23	M	57	Pellagra and urinary infection*	Inadequate	Good	27	176	3.0
24	M	54	Pellagra, scurvy and atrophic arthritis*	Inadequate	Good	61	146	1.7
25	M	53	Pellagra and Hodgkin's disease*	Inadequate	Fair	66	133	1.4

* These patients had plasma ascorbic acid values of less than 0.5 mgm. per 100 cc. on or shortly after admission.

† These patients also excreted during continuous intravenous administration of thiamin significantly less of the vitamin than did normal controls.

‡ After treatment the excretion of thiamin during continuous intravenous administration was also in the range of the normal control values.

TABLE III
Urinary excretion of thiamin by patients with organic heart disease

Case number	Sex	Age years	Type of heart disease	Degree of decompensation when tested	Thiamin content of previous diet	Dietary thiamin during test	Thiamin excreted in urine		Fraction of oral dose excreted in urine
							Before oral dose	After oral dose	
25	M	50	Hypertensive	++++	Inadequate	Fair (650 γ)	micrograms per 24 hours 33	micrograms per 24 hours 180	per cent 2.9
26	M	54	Hypertensive	+++	Suboptimal	Fair (500 γ)	19	63	0.9
27	M	54	Coronary	+++	Suboptimal	Good (780 γ)	64	348	5.7
28	M	66	Arteriosclerotic	++	Suboptimal	Fair	74		
29	M	37	Rheumatic	+	Suboptimal	Fair	59		
30	F	52	Hypertensive	+	Suboptimal	Fair	113	384	5.4
31	M	43	Hypertensive	0	Adequate	Good (700 γ)	104	850	14.9

improvement objectively when large doses of crystalline thiamin were given.

Patients with endocrine disorders By inference from work on experimental animals some of the diseases of endocrine origin have been suspected of being associated with abnormalities in utilization of thiamin. Himwich and associates (12) have accelerated the appearance of vitamin B deficiency in dogs by massive doses of thyroid.

Other workers (13, 14, 15, 16) have demonstrated that the amount of thiamin required to maintain weight gain in rats and pigeons is increased by experimentally induced hyperthyroidism. Frazier and Ravdin (17) have reported improved results by the use of yeast and thiamin in the preoperative preparation of hyperthyroid patients. Table presents the values for thiamin excretion on 7 patients with toxic goiter, 6 of whom show val-

TABLE IV
Urinary excretion of thiamin by patients with endocrine disorders

Case number	Sex	Age years	Clinical diagnosis	Thiamin content of previous diet	Dietary thiamin during test	Thiamin excreted in urine		Fraction of oral dose excreted in urine
						Before oral dose	After oral dose	
32	F	36	Exophthalmic goiter	Adequate	Good	micrograms per 24 hours 106	micrograms per 24 hours 1206	per cent 22.0
33	F	47	Exophthalmic goiter	Adequate	Good	56	750	13.9
34	F	36	Recurrent exophthalmic goiter	Adequate	Good	157	750	11.9
35	M	35	Toxic adenomatous goiter	Adequate	Good	149	1140	19.8
36	M	34	Exophthalmic goiter	Adequate	Good	188	1026	16.8
37	M	20	Exophthalmic goiter	Adequate	Good	195	732	10.7
38	M	47	Exophthalmic goiter	Inadequate for 2 1/2 weeks	Good (980 γ)	44	182	2.8
39	F	51	Myxedema	Suboptimal	Fair	18	230	4.2
40	F	52	Diabetes mellitus*	Adequate	Good (900 γ)	70	705	12.7
41	F	53	Diabetes mellitus*	Adequate	Good	144	846	14.0
42	F	67	Diabetes mellitus†	Adequate	Good	46	924	17.6
43	M	68	Diabetes mellitus†	Adequate	Good	127		
44	M	25	Diabetes mellitus†	Adequate	Fair	103	690	11.7
45	M	27	Diabetes mellitus†	Adequate	Good	300	1152	17.0

* No neurologic complaints. neurologic examination normal.

† Symptoms sensory disturbances and reflex changes of peripheral neuritis.

‡ No symptoms or sensory disturbances. Absent tendon reflexes in lower extremities.

well within the normal range. These patients had experienced the usual polyphagia of their disease and had taken diets of high caloric and vitamin content. Case 38, who showed definitely low urinary thiamin values, had also eaten well until anorexia developed about 18 days prior to the tests. He was extremely toxic on admission and went into thyroid crisis which terminated fatally on the seventh day of hospitalization. These findings suggest that in hyperthyroidism the usual increase in food consumption suffices to meet the increased requirement for thiamin, but that in the occasional patient with poor appetite or gastrointestinal disturbances a deficiency of this vitamin

may rapidly develop. The 1 case with classical myxedema studied showed low values for thiamin excretion, this patient had not eaten well prior to the test.

The steadily mounting experimental evidence that thiamin plays a role in the intermediary metabolism of carbohydrates (18) has aroused considerable interest in the status of diabetics with regard to this vitamin (19). Of particular interest is the relationship of "diabetic neuritis" to the peripheral neuritis of beriberi. All of 6 regulated diabetics studied showed excretory values in the normal range. These patients had all been on diets of adequate thiamin content before coming

TABLE V
Urinary excretion of thiamin by patients with gastro-intestinal disease

Case number	Sex	Age	Diagnosis	Thiamin content of previous diet	Dietary thiamin during test	Antacid medication during test	Thiamin excreted in urine		Fraction of oral dose in urine	Comment
							Before oral dose	After oral dose		
		years					micro-grams per 24 hours	micro-grams per 24 hours	per cent	
46	M	39	Marginal ulcer	Adequate	Good	CaCO ₃ 1 gram and Mg ₃ Si ₂ O ₈ 0.5 grams q 1 hour	99	792	13.9	2 years after gastro-enterostomy
47	M	25	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	34	720	13.7	Recent chronic hemorrhage
48	M	34	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	15	630	12.3	Recent chronic hemorrhage
49	M	58	Atrophic gastritis	Adequate	Good	Colloidal Al(OH) ₃ 6 cc q 1 hour	41	625	11.7	8 days after large hematemesis
50	M	29	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	32			
51	M	56	Gastric ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	31			X ray showed ulcer healed at time of test
52	M	66	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	63	594	10.6	Mild chronic pyloric obstruction
53	M	65	Duodenal ulcer	Adequate	Good	Colloidal Al(OH) ₃ 6 cc q 1 hour	30	492	9.2	Recent chronic hemorrhage
54	M	58	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams q 2 hours	46			
55	M	49	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 mgm q 1 hour	21	428	8.1	Just after relief of partial obstruction
56	M	56	Recurrent marginal ulcer	Adequate	Good	Colloidal Al(OH) ₃ 4 cc q 1 hour	43	373	6.6	19 years after gastro-enterostomy
57	M	56	Duodenal ulcer	Suboptimal	Good	Colloidal Al(OH) ₃ 8 cc q 1 hour	38	366	6.6	Recurrent hemorrhages for 10 years
58	M	47	Duodenal ulcer	Adequate	Good	Colloidal Al(OH) ₃ 6 cc q 1 hour	33	258	4.5	Tests on 4th and 5th hospital day
59	M	46	Duodenal ulcer	Suboptimal	Good	Colloidal Al(OH) ₃ 6 cc q 2 hours	26	123	1.9	4 days after recovery from alkalosis

TABLE V—Continued

Case number	Sex	Age	Diagnosis	Thiamin content of previous diet	Dietary thiamin during test	Antacid medication during test	Thiamin excreted in urine		Fraction of oral dose in urine	Comment
							Before oral dose	After oral dose		
		years					micrograms per 24 hours	micrograms per 24 hours	per cent	
60	F	38	Gastric carcinoma	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	32	590	11.2	Severe hemorrhage 5 weeks before
61	F	57	Duodenal ulcer	Adequate	Good	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	21	368	6.9	
62	F	29	Duodenal ulcer	Suboptimal	Good	Colloidal Al(OH) ₃ 8 cc. q 1 hour	24	215	3.8	
63	F	26	Gastric ulcer	Adequate	Good	Mg ₃ Si ₂ O ₈ 1 gram q 1 hour	46	726	13.6	30 cc. liquor hepatis daily for 1 week before test
64	M	38	Tuberculous peritonitis	Adequate	Good (700 γ)	CaCO ₃ 2 grams or MgCO ₃ 2 grams q 1 hour	34	390	7.1	Biopsy diagnosis
		10 weeks later		Excellent	High (1250 γ)	None	238	1800	31.2	Clinically quiescent for 6 weeks
65	M	49	Total gastrectomy	Adequate for 7½ weeks	Good (800 γ)	None	68	456	7.8	Carcinoma of stomach, resection 10 weeks before test
66	M	54	Pernicious anemia	Suboptimal	Fair	None	44	684	12.8	Mild posterolateral sclerosis
67	M	73	Pernicious anemia	Inadequate	Fair (600 γ)	None	12	324	6.2	Mild posterolateral sclerosis
68	M	53	Pernicious anemia	Inadequate	Fair (500 γ)	None	12	60	1.0	No neurological abnormality
69	M	55	Abdominal carcinoma	Inadequate	Low (300 γ)	None	50			Primary in stomach
70	M	47	Subacute toxic hepatitis	Suboptimal	Fair	None	59	216	3.1	Severe jaundice.
71	F	44	Advanced atrophic cirrhosis	Suboptimal	Fair (500 γ)	None	38	207	3.4	Biopsy diagnosis
72	M	53	Atrophic cirrhosis. Chronic alcoholism	Suboptimal	Fair (500 γ)	None	47			

to the hospital and none of them showed evidence of ketosis although 2 showed intermittent glycosuria and hyperglycemia. Balance studies with more careful attention to the state of control of the diabetes are expected to give more conclusive information.

Patients with gastro-intestinal disease Nineteen cases were studied while being treated by a modified Sippy regimen for peptic ulcer. Subsequently 3 of these were shown to have atrophic gastritis, tuberculous peritonitis and gastric carcinoma respectively rather than ulcer. These tests with one exception, were done after the patients had been under treatment for 2 or more weeks.

Most of the patients had been on good diets before hospitalization. Preceding and during the tests they ate their diets well. Calculations of these diets showed a daily intake of 700 to 900 micrograms of thiamin with a thiamin non fat-calorie ratio of approximately 1.0. Antacid medication was continued during the test period.

The results are presented in Table V. The values before the oral dose are definitely lower than those obtained from normal subjects except for 1 case; however, only 5 of the 16 given oral test doses excreted less than 7 per cent thereof. This type of response, a low value during the period when the sole source of thiamin is the

followed by excretion of a normal fraction of the oral dose, was observed in normally nourished subjects after subsisting for a few days on an experimentally deficient diet (2). Since the patients on the modified Sippy regimen are known to have ingested an adequate diet during the test, it is suggested that the antacid medication may be the factor responsible for the low urinary thiamin values. This may be due either to destruction of the dietary thiamin in the gastro-intestinal tract when alkali is given simultaneously, or to loss of the vitamin by way of the feces due to adsorption of the alumina gels or silicates. The results in Case 63 support this concept despite the fact that this patient had received whole vitamin B complex supplements for 1 week prior to the test, the excretion before the oral dose was only 46 micrograms, followed by a percentage excretion of the oral dose well in the normal range. Studies reported previously (1) have ruled out the possi-

bility that these results are due to destruction of thiamin by alkaline urine in the bladder.

A somewhat comparable situation might be anticipated in patients with achlorhydria. Case 65 was studied 10 weeks after a total gastric resection for carcinoma, he had ingested and retained a diet of adequate thiamin content for over 7 weeks prior to the test. He excreted less dietary thiamin than did the normal subjects, but the fraction of the oral dose excreted was not definitely low. One of 3 patients with pernicious anemia had values very similar to those patients receiving antacid medication, while the other 2 showed low values both before and after the oral dose.

The low excretory values obtained on the 3 cases of hepatic disease studied are of interest in view of the widely held opinion that the liver has an important role in the handling of thiamin (18). However, the results in these cases may be attributed to the preceding suboptimal thiamin in-

TABLE VI

Urinary excretion of thiamin by patients with various other diseases

Case number	Sex	Age	Clinical diagnosis	Thiamin content of previous diet	Dietary thiamin during test	Thiamin excreted in urine		Fraction of dose excreted in urine	Comment
						Before oral dose	After oral dose		
		years				micrograms per 24 hours	micrograms per 24 hours	per cent	
73	M	45	Brucellosis	Inadequate	Good (700 γ)	47	176	2.6	Mild peripheral neuritis
74	M	62	Carcinoma and osteomyelitis of mandible	Inadequate	Fair	38			
75	M	55	Chronic alcoholism	Adequate	Good	96			
76	M	48	Myalgia	Suboptimal	Fair (550 γ)	43	460	8.3	
77	M	46	Impotence	Adequate	Good	218	855	12.7	Unaffected by thiamin therapy N P N 100 to 75 mgm per cent
78	M	52	Progressive peripheral polyneuritis	Adequate	Good	208	1386	23.6	
79	M	55	Chronic glomerulotubular nephritis with uremia	Adequate	Good (700 γ)	23	165	2.8	
80	F	36	Chronic glomerulotubular nephritis	Suboptimal	Fair (570 γ)	43	445	8.0	
81	F	23	Neurocirculatory asthenia	Suboptimal	Fair	26	384	7.2	N P N normal
82	F	19	Hysteria	Suboptimal	Good (700 γ)	46	435	7.8	
83	F	42	Sciatic neuralgia	Adequate	Good	53	536	9.7	
84	F	55	Bronchopneumonia	Adequate	Good	115	1002	17.7	
85	F	20	Gastro-intestinal allergy	Adequate	Fair	85			Tested during convalescence
86	F	45	Periarthritis shoulder	Adequate	Good	154			
87	F	40	Migraine	Adequate	Good	172			
88	F	28	? retrobulbar neuritis	Adequate	Good	130	1090	19.2	
89	F	23	Anxiety state	Adequate	Good	200			

take as well as to an impairment of storage in the body

Patients with miscellaneous diseases The results obtained on 17 patients with various other diseases are presented in Table VI. The low values for thiamin excretion by Case 73 suggest that the mild peripheral neuritis which complicated his brucellosis might well be attributed to a thiamin deficiency, however, it was impossible to carry out therapy suitably controlled to rule out a toxic etiology. Case 79 with terminal nephritis and chronic nitrogen retention had eaten an adequate diet for 4 weeks before the test, the low urine values obtained may indicate that in renal insufficiency the kidney is unable to excrete thiamin. Among the other cases in this group there is apparent some correlation between the thiamin content of the preceding diet and the values for urinary thiamin excretion.

DISCUSSION

The correlation in the individual subject between the urinary excretion of thiamin before and after the oral test dose is concordant when judged by the above standards in 68 of the 75 cases in which both values were obtained excluding patients with achlorhydria and those receiving antacid medication. We have observed only 1 subject (Case 30, Table III) in whom a normal value for the 24-hour excretion was followed by excretion of an abnormally small fraction of the test dose. The remaining 6 subjects excreted a normal fraction of the test dose despite a preceding 24-hour value in the definitely low range. 4 of these were known to have eaten poorly on the day of the test. These results are interpreted as indicating that insufficient thiamin from dietary sources was available for absorption from the gastro intestinal tract on the day of the test, but that there was no significant depletion of the thiamin stores. Experimental evidence for this interpretation is presented elsewhere (2).

The results in the patients with achlorhydria and those receiving antacid medication suggest that in these cases diagnostic significance should be attached only to the fraction of the test dose excreted. They also indicate that factors in the gastro-intestinal tract, which affect the availability of thiamin for absorption may be of clinical sig-

nificance. Preliminary studies indicate that a significant reduction in urinary thiamin excretion follows the administration of antacid medications to patients on a constant dietary intake. Results of preliminary studies on the stability of thiamin *in vitro* in the secretions of the gastro-intestinal tract indicate that the vitamin is stable in achlorhydric and in normal gastric juice from its natural acidity to pH of 8. However significant losses occur in pancreatic juice and bile at the natural pH of 8, but not at pH 4.5. It is suggested that with gastric acidity neutralized or absent the contents of the small intestine may become alkaline enough to permit an abnormal destruction of thiamin.

In discussing standards of normality and deficiency it is important to bear in mind that low values for urinary thiamin can in themselves indicate no more than a depletion of the body stores of this particular food factor. From the data presented in the tables it is obvious that there is no particular level of urinary thiamin excretion at which signs and symptoms of thiamin deficiency appear. The clinical significance of the finding of thiamin subnutrition in a patient who presents none of the recognized clinical features of thiamin deficiency is uncertain. Before the physiologic alterations and clinical symptoms associated with the wide variety of diseases presented in this paper can be attributed to thiamin deficiency it will be necessary to evaluate the response of such cases to thiamin therapy under carefully controlled conditions or to demonstrate a specific biochemical dysfunction.

SUMMARY AND CONCLUSIONS

Under proper conditions the level of the urinary excretion of thiamin permits an objective determination of the state of thiamin nutrition in the human subject.

There is good correlation between the urinary thiamin values and the adequacy of the preceding diet with respect to this vitamin. The 24-hour urinary thiamin excretion in subjects whose previous dietary intake of thiamin had been adequate, and who ingested an adequate diet on the day of the test, was 90 micrograms or more in all males and above 60 micrograms in all females but one. Under similar conditions, the 24-hour excretion in

loblasts X-ray of the gastro-intestinal tract was normal. The Wassermann reaction was negative. Ten days following the first glycine test the patient experienced a transitory renal disorder. It was characterized by chills, temperature of 100°, many hyaline casts in the urine, and tenderness in the left kidney region. The urea clearance was 20 per cent of normal at that time. All evidence of this disorder disappeared after 48 hours, and no more attacks occurred during the 13 months that the patient was followed. The reticulocyte count rose to 124 per cent on the tenth day following the first injection of liver extract, and 70 cc. of liver extract were given during the 3 months between tests. Clinical

recovery was complete. At the time of the second test the red blood cell count was 4,980,000, hemoglobin 107 per cent. Urea clearance was 105 per cent of normal.

3 Patients with pernicious anemia (previously treated)

Case 5 E. McK., female, aged 50. Hospital Number 10221. Admitted on July 28, 1937, complaining of weakness, pallor, numbness and tingling of the arms and legs and ataxia (see Figure 3). One year previous to admission the patient had noted anorexia and difficulty in walking. She was treated with liver extract and a remission occurred, but therapy was discontinued and she was admitted in a relapse. She was a well developed, well nourished, rather pale woman. The lingual papillae were atrophic at the tip, the vibratory sense was absent, and sensation to light touch was diminished. The red blood cell count was 2,060,000, hemoglobin 43 per cent. Urea clearance was 100 per cent of normal. No free hydrochloric acid was present in the gastric juice even after the injection of histamine. The gastro-intestinal series was negative. After the first glycine test liver extract was administered and a reticulocytosis of 12 per cent occurred. The clinical remission was gradual but complete. At the time of the second test the red blood cell count was 4,320,000, hemoglobin 90 per cent. Urea clearance was 100 per cent of normal.

Case 6 M. K., female, aged 67. Hospital Number 10456. Admitted April 29, 1938, complaining of weakness, blurred vision, numbness, tingling of finger tips, and pallor (see Figure 3). Three years before the patient had noted gradually increasing weakness and pallor. Later blurred vision and numbness in the finger tips developed and liver extract was administered. Improvement followed, but therapy was discontinued and a relapse ensued. Lextron had been taken before admission without clinical effect. She was well developed but slender. In the retinal vessels numerous sclerotic areas were visible. The oral mucous membranes were normal and the vibratory sense was poor. The red blood cell

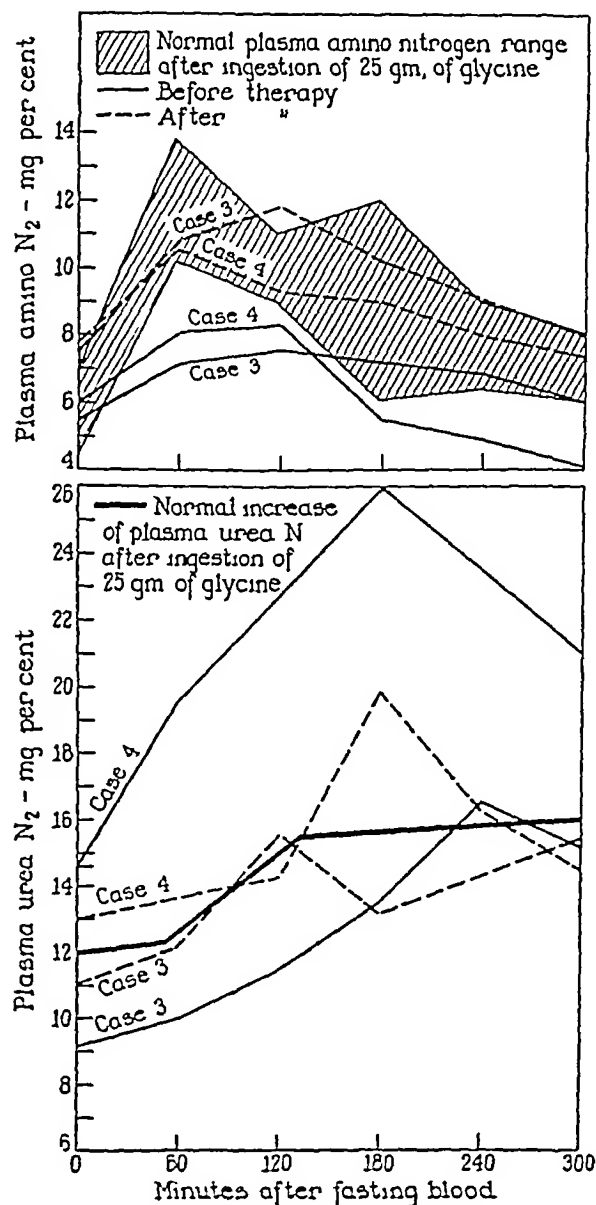


FIG. 2. PERNICIOUS ANEMIA, NOT PREVIOUSLY TREATED

FIG. 2 PERNICIOUS ANEMIA—NOT PREVIOUSLY TREATED
Before therapy

	Red blood cells	Hemoglobin	Color index	Total urea for mation	Urea clearance test	Liver function tests (urinary urobilinogen test Harrop, Barron and Quick tests)
		per cent		mgm.	per cent of normal	
Case 3	980,000	31	1.6	2714	180	65.0-15.0 mgm. urinary urobilinogen daily
Case 4	1,640,000	44	1.3	1750	23	3.9 per cent retention of bilirubin 2.5 grams of hippuric acid excreted in 4 hours
After therapy						
Case 3	3,700,000	76	1.0	2321	100	3.5 mgm. urinary urobilinogen daily
Case 4	4,500,000	107	1.0	2335	105	

count was 2,880,000, hemoglobin 63 per cent. Urea clearance was 60 per cent of normal. Bilirubin excretion test, 2.0 per cent retention sodium benzoate excretion 2.9 grams, daily urinary urobilinogen, 0.8 mgm. Four degrees of free hydrochloric acid were present after injection of histamine. The sternal bone marrow was hyperplastic and a differential count revealed an increased number of normoblasts and erythroblasts. After the administration of liver extract the reticulocyte count rose to 8.8 per cent. The clinical remission which followed was gradual but complete. At the time of the second glycine test the red blood cell count was 4,170,000 hemoglobin 87 per cent. Urea clearance was 90 per cent of normal.

Case 7 J. B., male, aged 40. Hospital Number 9757

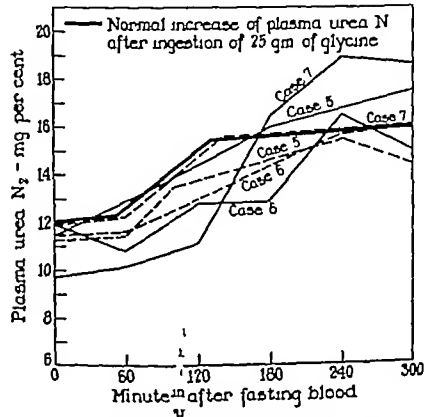
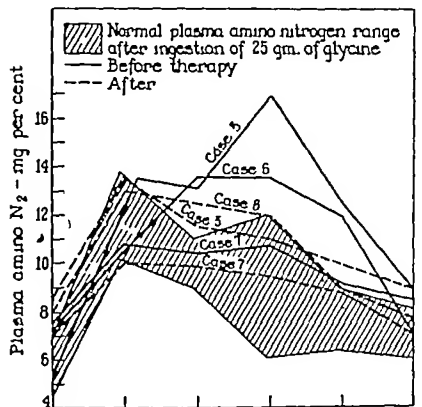


FIG. 3. PERNICIOUS ANEMIA, PREVIOUSLY TREATED

Admitted on February 11 1938, complaining of weakness, pallor shortness of breath, glossitis, and occasional paresthesias of the lower extremities (see Figure 3). The patient had had 2 induced remissions of his disorder before his admission. He was well developed, and well nourished, with a yellowish pallor of the skin. The tongue was diffusely reddened but the papillae were not atrophic. The vibratory sense was diminished. The red blood cell count was 2,470,000 hemoglobin 69 per cent. Urea clearance was 120 per cent of normal. Bilirubin excretion test, no retention sodium benzoate test, 3.2 grams daily urinary urobilinogen, 2.5 mgm. No free hydrochloric acid was present in the fasting gastric juice. The sternal bone marrow was hyperplastic, with an abnormally increased number of megaloblasts and erythroblasts. The gastro-intestinal series was negative. After the administration of liver extract there was a reticulocytosis of 16 per cent, and complete recovery followed. When the second glycine test was made the red blood cell count was 3,540,000 hemoglobin 84 per cent. Urea clearance was 100 per cent of normal.

4 Patients with pernicious anemia in induced remissions

Case 8 T. T., male, aged 60 Hospital Number 10355 Admitted February 1 1938, complaining of severe weakness and pallor. The patient had had 3 induced remissions before admission. He received 5 cc. of liver extract weekly for 6 months before the glycine test was made. At the time of the test the red blood cell count was 4,340,000 hemoglobin 90 per cent, and white blood cell count 6,900. The differential count was normal. Bilirubin excretion test, 6 per cent retention sodium benzoate test, 3.93 grams. Urea clearance was 100 per cent of normal.

Case 9 C. M., female, aged 71 Hospital Number 10085 Admitted March 6, 1937 complaining of weakness

FIG. 3. PERNICIOUS ANEMIA—PREVIOUSLY TREATED
Before therapy

	Red blood cells	Hemoglobin	Color index	Total urea for reaction	Urea clearance test	Liver function tests (urinary urobilinogen test, Harrop, Barron and Quick tests)
		per cent		mgm.	per cent of normal	
Case 5	2,060,000	42	1.0	1011	100	0.8 mgm. urinary urobilinogen daily
Case 6	2,880,000	63	1.1		60	2.0 per cent retention of bilirubin 2.9 grams of hippuric acid excreted in 4 hours
Case 7	2,470,000	69	1.4	2774	120	2.5 mgm. urinary urobilinogen daily 0.0 per cent retention of bilirubin 2.5 grams of hippuric acid excreted in 4 hours

After therapy

Case 5	4,320,000	90	1.0	2327	100	
Case 6	4,170,000	87	1.0	2774	90	
Case 7	3,540,000	84	1.4	2578	100	

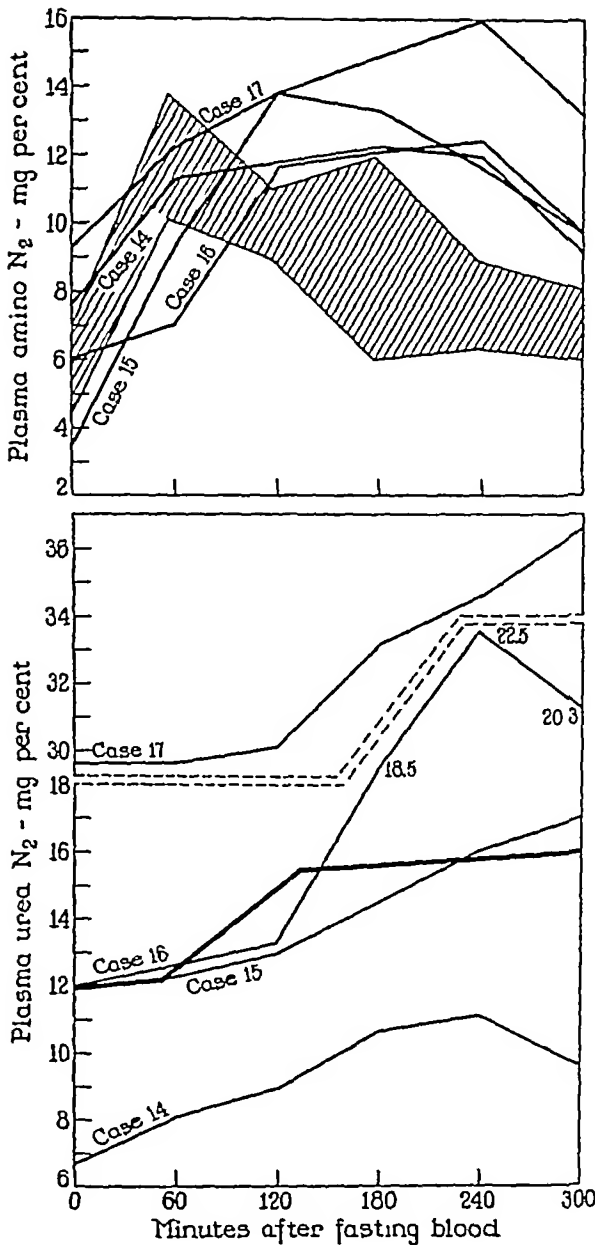


FIG. 6 ULCERATIVE COLITIS

patients they were delayed to between 120 and 240 minutes. The plasma urea nitrogen curves also had delayed peaks and 1 (Case 14) was below the normal level, whereas 2 (Cases 16 and 17) were definitely elevated. The urea clearance was 55 per cent of normal in Case 16 and 20 per cent of normal in Case 17. Presumably the elevations were due to renal insufficiency with retention of urea.

7 Patients with hepatic cirrhosis (see Figure 7)

This group was composed of 2 patients (Cases 18 and 19) who had clinical, laboratory, and finally postmortem evidence of advanced hepatic insufficiency with destruction of liver tissue. The plasma amino nitrogen curve in Case 18 had a delayed peak and in Case 19 it was flat but at a relatively high level. In both cases the plasma urea nitrogen curves were below normal.

DISCUSSION

Malabsorption of glucose and of fat has been established as a feature of tropical sprue, and evidence is available to suggest that glucose, at least, is not absorbed normally by patients with pernicious anemia. The results here presented indicate that the amino acid, glycine, also is poorly absorbed by patients with untreated sprue but after

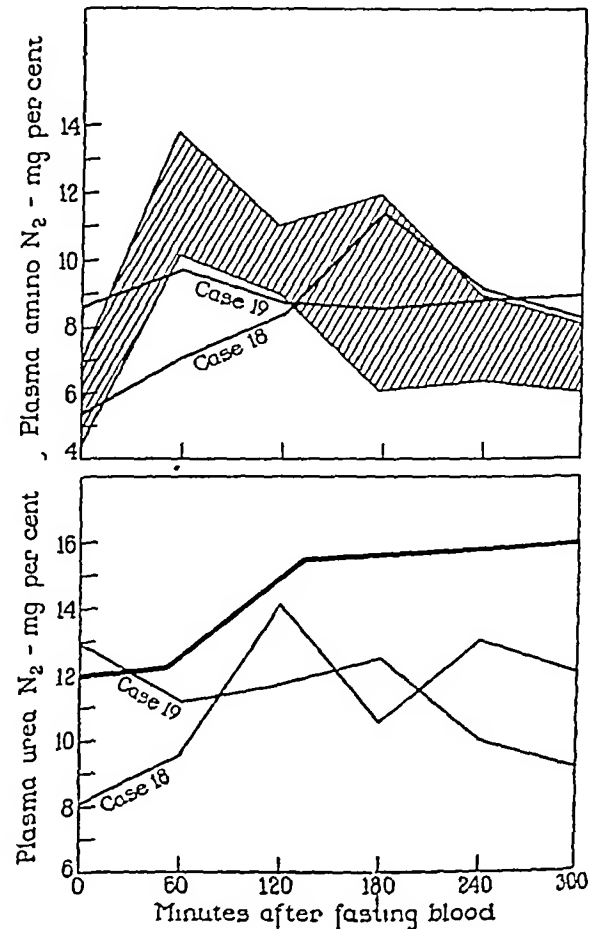


FIG. 7 HEPATIC CIRRHOSIS

the symptomatic improvement which follows the administration of liver extract the abnormality is no longer detectable. Furthermore, the low rates of formation and excretion of urea became normal after induced remission. The low curves for plasma amino and urea nitrogen observed before treatment can probably be attributed to poor absorption and not to simple loss of glycine from the bowel since the patients did not have diarrhea during the tests.

Once absorbed carbohydrates, fats and proteins have not been shown to be abnormally metabolized in sprue, indeed the results of glucose tolerance tests are normal even at the height of symptoms if the glucose is administered intravenously. It is possible however that the slow rates of urea formation before therapy by the patients here reported can be accounted for partially by some hepatic dysfunction in the metabolism of amino acids. In accord with this possibility is the fact that the results of 3 liver function tests were abnormal in both patients before treatment but became normal after the remissions were established.

In the 2 patients with pernicious anemia who had not been treated with liver extract previous to admission, the plasma amino nitrogen curves were also below normal and flattened. As in the cases with sprue, these results cannot be attributed to poor absorption because of diarrhea for both patients had normal bowel function. Either there was poor absorption in both groups because of some specific dysfunction of the gastrointestinal tract or the glycine was quickly deaminized. The latter assumption is unlikely since, if it were true the rates of urea formation should be rapid and thus was not the case. An elevated plasma urea nitrogen was found in 1 case (Case 4), presumably due to urea retention since only 1750 mgm of urea were formed and since the renal function test was but 28 per cent of normal. There was no clear evidence of hepatic dysfunction in this patient, although Fouts, Helmer, and Zervas (14) have shown that the results of the sodium benzoate test are somewhat abnormal in patients with pernicious anemia. In the other patient (Case 3) the plasma urea nitrogen curve was below normal and 2714 mgm of urea were formed, the urea clearance was 180 per cent and the rate of excretion of urinary urobilinogen was abnormally elevated (650 to 150 mgm. daily). In short, since no

case of sprue or untreated pernicious anemia presented evidence of abnormally increased deamination and since no glycine was lost from the bowel during the test it is assumed that the flat curves of plasma amino nitrogen indicate that absorption of glycine was impaired.

After therapy the curves became normal and by the same criteria it is assumed that the absorption of glycine improved. The fact that the amount of urea formed also became normal in both groups of patients is in accord with this assumption.

In the 3 tests made on patients with pernicious anemia in relapse following inadequate liver extract therapy 1 plasma amino nitrogen curve was normal and 2 were elevated. This fact indicates that the absorption of glycine was normal but the rate of deamination was possibly impaired in 2 cases. The results of renal and hepatic function tests were normal thus evidence being opposed to the presence of impaired hepatic deamination or renal excretion as a cause of the high amino acid levels. The peaks of the plasma urea nitrogen curves were delayed in 2 patients however, and in these the amount of urea formed was slightly less than normal (2774 mgm.) in one (Case 7) and much less than normal (1911 mgm) in the other (Case 6). The peak of the curve was more delayed in the latter patient than in the former. The plasma urea nitrogen curve was normal in the third patient (Case 5) but the amount of urea formed was not measured. From the plasma urea nitrogen levels it appears that a subnormal rate of deamination may be present in spite of good hepatic function as indicated by other tests. After adequate treatment the glycine curves became normal in all 3 patients.

The results suggest that relapse of previously treated pernicious anemia is attended by distinctly less disturbance of glycine absorption and metabolism than is shown by patients in their first attacks. It is noteworthy however, that patients of the relapse group were tested when their anemia was much less severe than was that of either of the cases in their first attack, or of the patients with sprue.

The possibility that ischemia of the gastrointestinal tract might play a role in the poor absorption of the amino acid by patients with low blood levels can be ruled out by the observation

made on 3 patients with severe refractory anemia. These had hemoglobin and erythrocyte levels as low as any of the patients with pernicious anemia or sprue, and yet their plasma amino nitrogen curves were essentially normal. Furthermore, the urea nitrogen curves were elevated in spite of normal urea clearances. Hence the absorption of glycine was probably even more rapid than the amino nitrogen levels would indicate.

Although, as previously mentioned, no patient had diarrhea during the period of the test it was still necessary to rule out more conclusively that symptom as a cause of malabsorption of glycine with resultant low curves of plasma amino nitrogen. Accordingly, the group of patients with ulcerative colitis and persistent active diarrhea was studied. In no instance was a low plasma amino nitrogen curve observed, indeed the levels were somewhat elevated, a fact supposedly due to a reduced rate of deamination. This supposition could not be proved, however, since in Cases 16 and 17 high plasma urea values were found also, a fact referable to abnormally low rates of excretion (urea clearance 55 per cent and 20 per cent of normal, respectively). For the same reason, the subnormal sodium benzoate test results do not supply valid evidence of hepatic dysfunction as a possible cause of low deaminizing power.

In the studies already discussed, some evidence of hepatic dysfunction was at hand in those patients with sprue and untreated pernicious anemia who seemed to show evidence of poor absorption. No such evidence was found in the patients with pernicious anemia in relapse, and it was accordingly necessary to test the absorptive ability of patients with advanced hepatic insufficiency from cirrhosis. These were the only control studies which indicated a definite impairment of absorptive power. One plasma amino nitrogen curve was flat and below normal and the other was low, although a rise did appear late in the curve of the test. The urea nitrogen curves also were abnormally low. This would be expected if the defect were one of absorption. Definite evidence of hepatic dysfunction was at hand in the results of other tests on these patients.

It is suggested that the abnormal results of the glycine tolerance tests in patients with sprue and

pernicious anemia in relapse are not dependent upon the associated diarrhea or anemia but possibly upon some hepatic dysfunction. The results of the glycine tolerance tests and hepatic function tests made on patients with hepatic cirrhosis were somewhat similar to those made on patients with untreated pernicious anemia and sprue. With adequate liver extract therapy the results of the tests in the latter groups of patients returned to normal or near normal levels but, as would be expected, this measure was ineffective in restoring normal hepatic function to patients in whom the majority of the liver cells had been replaced by fibrous tissue. The suggestion is also strong that abnormalities in the handling of glycine may be due to a lack of some constituent of liver extract, not the anti-pernicious anemia substance.

CONCLUSION

1 In 4 patients with untreated sprue and pernicious anemia the results of glycine tolerance tests suggested that glycine was absorbed from the gastro-intestinal tract more slowly than normal.

2 Evidence of this abnormality was not found in the same cases after the administration of liver extract.

3 Evidence of malabsorption was not demonstrable in patients with intractable diarrhea, severe refractory anemia or pernicious anemia in complete or partial remission, but was present in 2 patients with cirrhosis of the liver.

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pared with normal values of other investigations. Consequently, it is difficult to evaluate these results. Analysis of blood from twenty-five arthritic patients showed normal total sulfur, total sulfate and average inorganic sulfate content and no variation in the average reduced glutathione content following administration of sulfur.

In the small amount of literature dealing with sulfur metabolism in relation to arthritis, there is much that is contradictory and the data certainly do not support the numerous far-reaching and diverse statements that are made concerning altered sulfur metabolism, sulfur deficiency, and the like, in patients with arthritis.

The rationale for the administration of sulfur to patients with arthritis is based primarily on the following claims. (1) It is said that a deficiency of sulfur exists in chronic arthritics, as evidenced chiefly by a lowered content of cystine in fingernails (8), and by a reduced sulfur content of articular cartilage (9). (2) It is reported that free indole is often found in the urine of arthritics and, since oxidized sulfur is used in the detoxification of indole, it is *inferred* that an insufficient supply of sulfur exists for the complete detoxification of this substance or that, due to some metabolic fault, available sulfur is not used normally for this purpose.

Because of the disagreement concerning the value of sulfur therapy and because there is insufficient knowledge of the metabolism of sulfur in arthritics, the investigations herein reported were conducted. The studies were planned primarily to answer two questions. (1) Is there a deficiency of sulfur or an abnormality of sulfur metabolism in patients with chronic arthritis? (2) If there is such a deficiency or abnormality, does the administration of sulfur in various forms benefit or correct either? No attempt was made to evaluate sulfur therapy on the basis of *clinical* changes in the patients studied.

PROCEDURE

Subjects

The metabolism studies were made on patients with various types of arthritis and on normal individuals (see Table I). Four patients had rheumatoid arthritis in different stages of the disease. Three were males, one was a female, their ages ranged from twenty-six to forty

years. The disease had existed only three months in O J at the time the investigation began. Many of the affected joints were acutely inflamed and fever of one to two degrees occurred daily throughout the period of study. G Mc. was in the subacute stage of the disease. Several joints showed signs of active inflammation, in others the inflammation had subsided, at times he had slight fever. S K., who was in the early chronic stage of the disease, had had arthritis for twenty months when these studies began. All involved joints showed considerable periarticular swelling, in some joints inflammation was slightly active, while in others only the residual changes of previous inflammation existed, especially contractures and impairment of motion due to cartilage destruction. C B was incapacitated because of arthritis which he had had for six years. There were no clinical signs of active joint inflammation but the erythrocyte sedimentation rate was elevated. His chief difficulties were marked limitation of joint motion, multiple flexion contractures and pain in the weight-bearing joints when standing. He represented the late chronic stage of the disease when joint damage from previous inflammation was of paramount importance.

The three patients with spondylitis rhizomelica varied in age from eighteen to thirty-one years, all were males. R. H., who was in the early stage of spondylitis, had symptoms only in the low dorsal and lumbar spine and sacro-iliac joints. Pain was the outstanding symptom, limitation of motion was only moderate and due chiefly to muscle spasm and pain. S G had had symptoms for six years. There were pain and immobility of the dorso-lumbar spine. Roentgenograms showed obliteration of sacro-iliac joints and beginning calcification of longitudinal spinal ligaments. J S had had spondylitis for twelve years, he no longer had pain but was almost completely incapacitated because of rigidity of the entire spine in severe kyphosis and ankylosis of both hips. Extensive calcification of the ligaments was demonstrated by roentgenograms.

Two patients with typical degenerative disease of the joints ("hypertrophic," "osteo" arthritis) were studied. One, D M, a female, sixty-one years, had typical bilateral malum coxae senilis and no other clinical arthritis. The other, G M, a male, sixty-one years, had typical clinical and roentgenological changes in many of the distal phalangeal finger joints, a few proximal phalangeal finger joints and in both knees. The disease had existed for ten and six years, respectively, in these patients.

These differences in sex, age and clinical characteristics of the disease clearly show that these patients represent all of the important stages of each of the types of arthritis studied. None of the arthritics had any other disease which in any known way would affect the sulfur metabolism.

The five control subjects were healthy persons varying in age from twenty-three to fifty-one years. One was a female. D Mc. was an unemployed laborer, the others were students.

TABLE I

Average twenty-four hourly urinary excretion of nitrogen and sulfur in all subjects during control periods

Subject	Sex	Age	Body weight	Duration of illness	Nitrogen excretion	Urinary sulfur excretion						N/S ratio			
						Total sulfur	Total sulfate sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur		
			pounds	months	grams	mgm.	mgm.	per cent of total	mgm.	per cent of total	mgm.	per cent of total	mgm.	per cent of total	
Normals															
W A.	M	27	165		9.16	757	622	(82)	567	(75)	55	(7)	135	(18)	12.04
D Mc.	M	51	159		8.90	711	594	(84)	548	(77)	46	(6)	116	(16)	12.52
H M	M	25	111½		7.42	640	505	(79)	473	(74)	33	(5)	135	(21)	11.60
M H	F	28	140		7.53	621	490	(79)	458	(74)	32	(5)	131	(21)	12.12
E W	M	23	179		8.26	680	554	(81)	522	(76)	28	(4)	130	(20)	12.13
Average			151		8.25	682	553	(81)	518	(75)	39	(5)	129	(19)	12.08
Rheumatoid arthritis															
G Mc.	M	26	154	6	9.01	695	558	(80)	490	(70)	69	(10)	136	(20)	12.96
O J	M	49	146	3	8.98	667	552	(83)	490	(74)	62	(9)	114	(17)	13.46
S K.	F	26	110½	20	7.29	540	478	(88)	424	(78)	52	(10)	64	(12)	13.50
C B	M	50	144½	72	8.14	698	540	(77)	476	(68)	62	(9)	159	(23)	13.10
Average			139		8.35	650	532	(82)	470	(73)	61	(9)	118	(18)	13.09
Hypertrophic arthritis															
G M	M	61	150	72	8.12	661	524	(79)	476	(72)	48	(7)	137	(21)	12.28
D M	F	61	125	120	7.37	623	526	(84)	442	(71)	84	(12)	96	(15)	11.83
Average			137		7.75	642	525	(82)	459	(72)	66	(10)	117	(18)	12.06
Spondylitis rheumatica															
R. H	M	18	110	36	6.23	520	411	(80)	363	(70)	48	(9)	108	(20)	11.98
S G	M	30	148	96	8.28	621	516	(83)	460	(74)	56	(9)	105	(17)	13.33
J S	M	31	130	144	7.70	599	501	(83)	451	(75)	50	(8)	98	(16)	12.85
Average			129		7.40	580	476	(82)	425	(73)	51	(9)	104	(18)	12.72

Plan of study

All subjects were fed identical diets except for the addition of butter cream sugar or mayonnaise to some in order to provide sufficient energy to prevent weight loss in larger and more active subjects. This was arranged so that the sulfur intake would be the same in all cases. Three menus were arranged for successive days these were rotated in order throughout the study. The protein content of the diet was kept low 60 grams, so that the intake of sulfur (source of which is chiefly protein) would be relatively small. In this way the basal excretion of sulfur would be small and changes in sulfur elimination during periods of sulfur therapy would be more sharply contrasted. The dietary protein was sufficiently high so that all subjects were in positive nitrogen balance.

The meals for all subjects were quantitatively prepared in the special metabolism kitchen and were served to the patients on the wards where they resided throughout the period of study. Control subjects lived outside of the hospital and ate all their meals in the metabolism dining room. Each subject drank a fixed amount of distilled

water daily. All of the food was eaten each day and vomiting or diarrhea did not occur.

After four to seven days were allowed for adjustment to the diet, all urine was saved in twenty four hourly collections and preserved with toluene. Urine obtained in this way was analyzed for total nitrogen, total sulfur total sulfate sulfur inorganic sulfate sulfur and by difference the conjugated sulfate sulfur and organic sulfur were calculated. Complete collection of urine was assured by daily creatinine analyses.

After a period of study (four to twelve days) when the diet provided the sole intake of sulfur various experiments were conducted. Sulfur was administered in different forms and by different routes, but similarly to patients and controls and changes in urinary sulfur were measured. Thymol was fed to some of the subjects to test the ability to conjugate inorganic sulfur. Experimental periods were always separated by many days of "control" when no medication was given. A terminal control period ended the investigation. The average daily urinary sulfur excretion during experimental periods was contrasted with the average daily excretion during control periods.

Finger-nail clippings were analyzed for cystine before the administration of sulfur and at approximately monthly intervals thereafter for three to seven months. The urine of eight of the arthritics and three of the controls was analyzed for indole daily.

Chemical methods

Urinary sulfur was partitioned according to the technique of Folin (10). Total sulfur was determined by the method of Denis-Benedict (11) and total nitrogen by the usual macro-Kjeldahl procedure. Cystine was determined by the Sullivan method as modified by Ros-souw and Wilken-Jorden (12), creatinine by the method of Folin (13) and indole by the procedure of Forbes and Neale (14) controlled by analysis of pure indole and a water blank to insure against false positive results.

EXPERIMENTAL DATA

Sulfur is eliminated almost entirely in the urine. A relatively small and fixed amount passes through the skin (15) and none is excreted by the bowel except as hydrogen sulfide which is formed by bacteria in the intestinal tract. Thus sulfur metabolism can be accurately studied by a complete analysis of urine, the small amount of sulfur eliminated by other routes was not measured.

In order to compare the sulfur and nitrogen metabolism of patients with arthritis and normal individuals, the average daily excretion of nitrogen and urinary sulfur distribution during control periods was determined. The number of control days varied in different subjects from fourteen to twenty-five. These data appear in Table I.

It should be noted that the total elimination of sulfur is essentially the same in the controls and all arthritics, although it is slightly higher in the controls as would be expected because of their larger size and greater activity. The per cent of sulfur eliminated as total sulfate, as inorganic sulfate and as organic sulfur is practically identical in all groups. The conjugated sulfate sulfur averages 9 or 10 per cent in different groups of arthritics and 5 per cent in the control subjects. It is this conjugated sulfate fraction which indicates the amount of sulfur combined with phenolic substances such as indole. The differences in the excretion of conjugated sulfate sulfur shown in Table I may be of no importance since the amount of sulfur eliminated as conjugated sulfate in *normal* individuals has been found to vary from 3 to 15 per cent of the total urinary sulfur. If these differences are significant, they certainly indicate

no deficiency in the available supply of sulfur and no impairment in the use of sulfur for this means of detoxification by the arthritic patient. The average N/S ratio varies only slightly in the different groups of subjects, we can attribute no significance to this slight variation.

From the data presented in Table I the following significant conclusions are drawn:

No important difference in the urinary sulfur distribution of patients with rheumatoid arthritis, hypertrophic arthritis, spondylitis rhizomelica, and normal individuals exists unless it be the slightly higher per cent of sulfur eliminated as conjugated sulfate by the arthritics. If this difference is significant, it certainly indicates *no deficiency in sulfur available for detoxification, and no impairment in this detoxifying function in arthritics.*

The effect of colloidal sulfur injected intravenously

To study the effect of the intravenous and intramuscular injection of colloidal sulfur, a preparation called "Sulisocol"^{*} was used. It was prepared so that one cubic centimeter contained 10 mgm of sulfur. Our analyses of this preparation showed that it contained no nitrogen and no organic material. This preparation was selected in order that we might study the effect of injection of sulfur alone, without simultaneously injecting protein or any other substance which might affect the sulfur metabolism. In all subjects, the colloidal sulfur was injected intravenously over a period of three days as follows. On the first day, 20 mgm were injected in the morning, 20 mgm in the evening, on the second and third days 50 mgm were injected both morning and evening. Thus, according to the statement of content on the medicament 250 mgm of sulfur were injected during the three-day period. (By our analysis it was found that we actually injected 260 mgm of sulfur.) In control subject E. W. one 50 mgm injection was omitted, and he was given only 208 mgm during the three-day period. The medicine was always injected slowly (at the rate of 1 cc per minute). Four controls and all the arthritis subjects were studied in this way. The experiment was carried out twice on the patients with

^{*} Kindly supplied for this study by The Drug Products Company, Long Island City, New York.

rheumatoid arthritis. Fever did not occur after any injections and no undesirable effect was observed.

The large amount of sulfur injected when the dietary source of sulfur was low produced a definite increase in excretion of sulfur and was completed in the majority of cases within twenty four hours after the last day of injection. The results of intravenous injections of colloidal sulfur appear in Table II. In this and similar tables to follow the effect of the administration of sulfur is measured by determining the amount of total sulfur and of each sulfur fraction excreted in the urine in excess of the average excretion during the control periods. If less sulfur was eliminated during the experimental period than the average during the control periods the value appears as a negative quantity.

Examination of Table II shows that in normal subjects the sulfur excretion increased from 76 to 135 per cent of the amount of sulfur injection

TABLE II
The effect of colloidal sulfur injected intravenously

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods							
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur		Organic sulfur	
		mgm.	per cent of added sulfur	mgm.	per cent of total	mgm.	per cent of total	mgm.	per cent of total
Normals:									
W. A.	250	198	(79)	159	(80)	-8	(-4)	48	(24)
D. M.	250	244	(91)	132	(33)	-36	(-15)	144	(59)
H. M.	250	252	(135)	224	(89)	-4	(-1)	28	(10)
E. W.	250	237	(114)	153	(64)	30	(13)	54	(24)
Average.	250		(105)		(77)		(-5)		(30)
Rheumatoid arthritis:									
G. M.	250	308	(163)	224	(73)	-4	(-3)	84	(26)
	250	458	(180)	308	(85)	12	(4)	144	(34)
O. J.	250	304	(117)	256	(84)	4	(1)	48	(15)
	250	478	(183)	330	(71)	0	(0)	140	(29)
S. K.	250	196	(73)	204	(70)	12	(5)	9	(4)
	250	290	(77)	218	(106)	-20	(-10)	8	(13)
G. B.	250	320	(200)	300	(58)	-64	(-19)	258	(55)
	250	334	(124)	320	(93)	-64	(-31)	73	(22)
Average.	250	312	(141)	270	(85)	-15	(-4.5)	98	(23)
Hypertrophic arthritis:									
G. M.	250	300	(118)	258	(90)	-28	(-9)	56	(19)
D. M.	250	322	(128)	316	(95)	-4	(-1)	0	(0)
Average.	250	316	(123)	292	(93)	-12	(-4)	30	(11)
Spondylitis rheumatica:									
S. G.	250	283	(110)	225	(79)	18	(5)	45	(16)
J. B.	250	498	(191)	238	(88)	122	(77)	76	(15)
	250	424	(164)	240	(87)	53	(12)	123	(21)
Average.	250	401	(185)	248	(85)	67	(15)	82	(21)

The arthritics eliminated from 75 to 200 per cent of the injected sulfur. In all but one arthritic (S. K.) the total sulfur excretion increased by amounts much greater than the amount of sulfur injected. The excess sulfur was eliminated chiefly as inorganic sulfate in both the controls and arthritics. The conjugated sulfur which it must be remembered is the form in which sulfur is eliminated when it is conjugated with phenolic substances changed insignificantly or was actually less than during control periods in all arthritics except one with spondylitis (J. S.). No important increase in elimination of conjugated sulfur occurred in any of the patients with rheumatoid or hypertrophic arthritis. In only three of the ten observations made on these patients was there any noticeable increase in conjugated sulfur excretion and in each of these instances the increase was much less than that occurring in one control subject. The average change in conjugated sulfur excretion in these groups of arthritics was negative. In only one of the three observations made on the two subjects with spondylitis, and thus in only one of a total of thirteen observations on arthritics, was there a greater increase in excretion of conjugated sulfate than occurred in the control subjects. It did not occur again when the patient was studied later.

The organic sulfur excretion did not change consistently. There was no difference between the arthritics and control subjects in this sulfur fraction. (It should be noted that through the entire study greatest changes in organic sulfur excretion occurred when the excretion of conjugated sulfur changed in the opposite direction.)

The following conclusions concerning the effect of intravenous injection of colloidal sulfur are drawn. The effect of colloidal sulfur injected intravenously is essentially the same in arthritics and in controls. Since the sulfur excretion increased by amounts greater than the amount injected, certainly the intravenous administration of colloidal sulfur would not benefit or prevent a sulfur deficiency if one existed. Since no significant increase in conjugated sulfur excretion occurred except in one of the thirteen observations made on eight arthritics, detoxification cannot be an important benefit resulting from the injection of colloidal sulfur.

*The effect of colloidal sulfur injected
intramuscularly*

Intramuscular injections of the same preparation of colloidal sulfur were given in the same amounts and according to the same schedule as in the intravenous studies. The results were observed in a similar way and appear in Table III. By our analysis it was found that we injected 270 mgm of sulfur in the three-day period instead of 250 mgm, which was the amount injected according to the statement of content on the medication. In two subjects W A (control) and D M (hypertrophic arthritis) the medicine caused moderate pain at the site of injection and, consequently, injections were stopped after 162 mgm had been given. No undesirable effects were noted, fever did not occur. Intramuscular injections were given to three controls and to the same arthritics that were formerly studied with intravenous injection of sulfur.

Examination of Table III shows that *all* subjects, arthritics and controls, had increased excretion of sulfur during the period of intramuscular injections, in fact, an excretion even greater than the amount of sulfur injected. Here again, the excess sulfur excretion was chiefly inorganic sul-

fate, as was the case when colloidal sulfur was given intravenously. In order to determine whether muscle destruction might account for this increased sulfur excretion, a non-sulfur-containing fluid was injected intramuscularly in one of the control subjects. Five cubic centimeters of calcium levulinate were injected on one day and ten cubic centimeters on the following day. No change in the excretion of nitrogen, total sulfur or any of its fractions occurred, indicating that muscle catabolism was not responsible for the increased sulfur excretion.

When colloidal sulfur was injected intramuscularly the excretion of conjugated sulfate varied slightly in different subjects, but in only one of the eight arthritics studied was this sulfur fraction increased by an amount greater than was observed in normal control subjects. This same patient showed the greatest *negative* change in the excretion of conjugated sulfate when colloidal sulfur was injected intravenously (see Table II). At no time during this study did this patient have indoluria. It should be noted further in this regard that (J S) the patient with spondylitis, who was the only arthritic to excrete more conjugated sulfate than controls when colloidal sulfur was given intravenously (see Table II), had one of the greatest *negative* changes in this fraction of urinary sulfur during the intramuscular studies. The differences in conjugated sulfate excretion in the case of each of these arthritics to whom attention has been directed are unimportant. No consistent change in the excretion of organic sulfur occurred.

From this study we conclude *Without exception the excretion of sulfur increased by amounts greater than the amount of sulfur injected intramuscularly, thus tending to create rather than prevent a deficiency of sulfur. With the possible exception of one patient, no important change in the excretion of conjugated sulfate occurred.*

The effect of colloidal sulfur given orally

Two normal subjects and the four patients with rheumatoid arthritis were given Mulford's "sulphocol," a preparation of colloidal sulfur designed for oral use. Each subject ingested six capsules in three equal doses during one day, providing 276 mgm of sulfur (our analysis). The results appear in Table IV.

TABLE III
The effect of colloidal sulfur injected intramuscularly

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods					
		Total sulfur		Inorganic sulfate sulfur		Conjugated sulfate sulfur	
		mgm.	per cent of added sulfur	mgm.	per cent of total	mgm.	per cent of total
Normals:							
W A.	162	460	(294)	320	(70)	-28	(-6)
D M.	270	294	(109)	192	(65)	36	(12)
E W.	270	330	(122)	400	(121)	35	(10)
Average			(171)		(85)		(8)
Rheumatoid arthritis:							
G M.	270	480	(175)	355	(74)	-75	(-16)
O J.	270	628	(233)	432	(69)	32	(5)
S K.	270	512	(190)	460	(94)	36	(7)
C B.	270	396	(147)	220	(55)	68	(17)
Average	270	504	(157)	122	(73)	15	(3)
Hypertrophic arthritis:							
G M.	270	305	(115)	260	(89)	-5	(-4)
D M.	162	246	(152)	150	(61)	-15	(-7)
Average			(133)		(75)		(-5)
Spondylitis rheumaticus:							
S G.	270	345	(128)	245	(71)	-40	(-12)
J S.	270	435	(161)	253	(66)	-55	(-13)
Average	270	390	(145)	265	(65)	-43	(-12)

TABLE IV
The effect of colloidal sulfur orally

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods			
		Total sulfur	Inorganic sulfate sulfur	Conjugated sulfate sulfur	Organic sulfur
	mgm.	per cent of added sulfur	mgm. per cent of total	mgm. per cent of total	mgm. per cent of total
Normals					
W. A.	275	247 (89)	220 (80)	14 (5.7)	13 (5.3)
D. M.	275	275 (100)	246 (89)	10 (4)	19 (7)
Average	275	212 (77)	203 (74)	15 (5)	57 (21)
Rheumatoid arthritis:					
O. M.	275	275 (100)	208 (75)	18 (7)	52 (19)
O. J.	275	231 (84)	235 (85)	3 (1)	-12 (-4)
R. K.	275	228 (83)	234 (86)	0 (0)	-1 (0)
C. B.	275	187 (68)	173 (63)	-13 (-5)	-9 (-3)
Average	275	222 (81)	210 (77)	3 (1)	9 (3)

The sulfur excretion changed in essentially the same way in the arthritics and the controls. The total sulfur excretion increased by amounts nearly equal to the amount given. When the excess excretion was less than the sulfur medication the difference can reasonably be accounted for by incomplete absorption and by elimination of sulfur by the bowel in the form of hydrogen sulfide. The added sulfur was eliminated chiefly as inorganic sulfate. In none of the subjects was there a significant increase in the excretion of conjugated sulfate or organic sulfur. The medication caused no discomfort. Its effect was over inside of twenty-four hours after administration.

From this study the following conclusions are drawn. Colloidal sulfur can be safely given orally. When so administered it is quickly eliminated almost entirely as inorganic sulfate. It is not used for conjugation and excreted as ethereal sulfate. Colloidal sulfur is metabolized and excreted in the same way whether given intravenously intramuscularly or orally thus indicating no advantage in parenteral use of the drug.

The effect of sodium thiosulfate given orally

Since elemental colloidal sulfur is eliminated in the urine chiefly as oxidized sulfur we wished to determine the effect of an inorganic sulfur-containing compound given orally. Sodium thiosulfate was used because relatively large amounts of this salt can be given without producing diarrhea.

The same subjects on whom the effect of oral colloidal sulfur was studied, and also one patient with spondylitis rhizomelica and two patients with hypertrophic arthritis were given sodium thiosulfate. In every case two grams of the salt were given in capsules in one dose after breakfast. The sulfur intake was thus increased by 810 mgm.

Table V shows the results are similar in arthritics and controls. The excess urinary sulfur excretion was less than the amount given. At first it might be thought that this represents retention of sulfur, however the fact that the control subjects showed precisely the same results as all of the arthritics would indicate that this is not the case. The difference can very reasonably be accounted for by the elimination as hydrogen sulfide gas by the bowel also some of the salt may not have been absorbed. As in the case of previous studies the excess sulfur in the urine was excreted almost entirely as inorganic sulfate, no significant change occurred in conjugated sulfate excretion. The effect was completed inside of twenty-four hours after ingestion of the salt.

This study shows that Sulfur given orally as sodium thiosulfate is quickly excreted almost entirely as inorganic sulfate by arthritics and controls and none is used for detoxification.

TABLE V
The effect of 2 grams of sodium thiosulfate orally

Subject	Added sulfur intake	Sulfur excretion in excess of average during control periods			
		Total sulfur	Inorganic sulfate sulfur	Conjugated sulfate sulfur	Organic sulfur
	mgm.	mgm. per cent of added sulfur	mgm. per cent of total	mgm. per cent of total	mgm. per cent of total
Normals					
W. A.	810	453 (56)	414 (51)	12 (1.7)	10 (1.3)
D. M.	810	573 (71)	524 (65)	-5 (-1)	50 (6)
Average	810	505 (62)	460 (57)	3 (0.4)	33 (4)
Rheumatoid arthritis:					
O. M.	810	506 (63)	454 (56)	4 (0.5)	36 (4)
O. J.	810	364 (45)	365 (45)	-7 (-1)	4 (0.5)
R. K.	810	460 (57)	441 (55)	1 (0.1)	19 (2)
C. B.	810	484 (60)	534 (66)	-25 (-3)	-14 (-2)
Average	810	476 (59)	472 (58)	-4 (-0.5)	11 (1)
Hypertrophic arthritis:					
G. M.	810	256 (32)	214 (27)	23 (3)	19 (2)
D. M.	810	466 (58)	466 (58)	-14 (-2)	13 (2)
Average	810	411 (51)	390 (48)	5 (0.6)	17 (2)
Spondylitis rhizomelica, R. G.	810	536 (66)	503 (62)	-15 (-2)	49 (6)

The effect of thymol administered orally

Even though the excretion of sulfur during control periods indicated no impairment of detoxification by conjugation of potential toxins with sulfur, and though sulfur administered in different ways was not used for this purpose, we wished to study this detoxifying mechanism further because of the prevalent belief that there may be a fault in the metabolism of sulfur which interferes with sulfur conjugation. Figure 1 indicates the manner in which phenolic substances are combined with sulfuric acid and excreted. Indole is regarded by some investigators (16) as an important factor in the causation of arthritis. We wished to determine the ability of arthritics to eliminate a phenolic substance quantitatively administered. We chose thymol as the test chemical because it is non-toxic and can be safely given orally in amounts sufficient to make an adequate test (17).

One-half a gram of thymol was given in capsules to three control subjects and to all of the arthritic patients studied. This medication was given in one dose after breakfast. If all the thymol were eliminated as ethereal sulfate, 107 mgm of sulfur would be required for the conjugation. If impairment of this detoxifying mechanism existed in any of the arthritics, the increase in excretion of conjugated sulfate during the period of thymol administration would be significantly less

than that of the control subjects studied in the same way.

The results of this investigation are shown in Table VI. As would be expected, the total sulfur excretion was not importantly affected. The excretion of conjugated sulfate was significantly increased in all subjects and the inorganic sulfate elimination was very definitely decreased in all subjects. Thus, it appears that thymol was conjugated with sulfur that otherwise would have been eliminated chiefly as inorganic sulfate. No consistent change in organic sulfur was noted. It was disappointing to us that this test was so indelicate. The amount of conjugated sulfate excreted varied considerably in the different subjects. The widest range was noted in the control group. In each group of arthritics the average conjugated sulfate excretion was increased slightly less than it was in the control group. This is due to the one high value in control W. A. which was 125 per cent of the theoretical total. It is obviously impossible to have more than 100 per cent conjugation of thymol. This high value in W. A. is undoubtedly due to a *basal excretion* of conjugated sulfate considerably greater than the average on the experimental day when a high percentage of thymol was also conjugated. It is unfortunate that this occurred for it falsely suggests a difference in the two groups which undoubtedly does not exist. It is most important to note that t

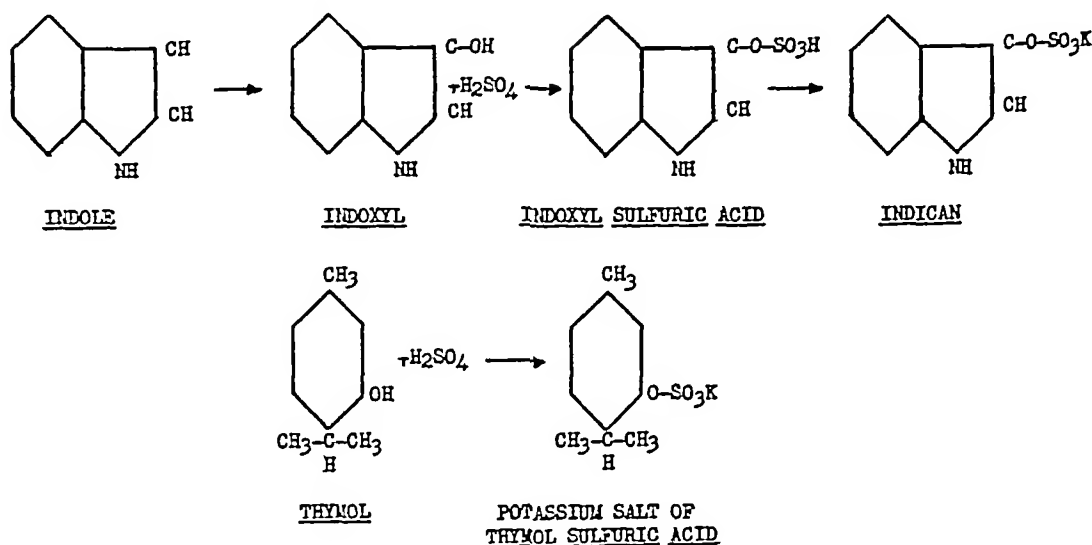


FIG. 1. SHOWING THE MANNER IN WHICH PHENOLIC COMPOUNDS ARE CONJUGATED WITH SULFURIC ACID

TABLE VI

Effect of the ingestion of 0.5 gram of thymol

Subject	Sulfur excretion compared to average during control periods			
	Total sulfur	Inorganic sulfate sulfur	Conjugated sulfate sulfur	Organic sulfur
	mgm.	mgm.	mgm. per cent of theoretical total*	mgm.
Normals				
W A.	-64	-80	140 (125)	-124
D Mc.	-1	-48	66 (62)	-21
E W	0	-83	40 (43)	30
Average	-21	-70	82 (77)	-38
Rheumatoid arthritis				
G Mc.	-16	-86	103 (96)	-33
O J	-29	-131	55 (51)	48
S K.	-2	-66	51 (48)	14
C B	-3	-112	50 (47)	60
Average	-12	-99	65 (61)	22
Hypertrophic arthritis				
G M	-65	-125	49 (45)	11
D M	70	-14	58 (54)	26
Average	3	-55	54 (49)	18
Spondylitis rhizomelica				
S G	-25	-92	43 (40)	24
J S	67	-65	95 (89)	37
Average	21	-78	69 (64)	30

*0.5 gram thymol requires 107 mgm. sulfur for complete conjugation

excretion of conjugated sulfate by all arthritics was within the range of excretion of this fraction of sulfur in the control subjects.

This study leads to the conclusion that *No deficiency of sulfur available for conjugation with phenolic substances exists in arthritics and no impairment of this detoxifying mechanism is evident in arthritics*

In this connection it is interesting to note that the urine collected from six of the eight arthritics and from two of the controls was tested daily for indole, and the results were entirely negative each day on all subjects but one J S a patient with spondylitis who had slight traces of indole in the urine on only two successive days. It is interesting that this indoluria occurred after the patient had received large amounts of sulfur and actually during a test period when colloidal sulfur was being injected intravenously

Finger nail cystine studies

The cystine content of finger nails of all subjects was determined before any sulfur preparations were administered and at approximately monthly intervals thereafter usually for three or more months. The data appear in Table VII. To better evaluate the results obtained in these subjects, analyses of finger nails of other persons were made and are tabulated in Table VIII.

Normal individuals are reported by Sullivan and Hess (8) to have finger nail cystine of from 10.28 to 13.02 per cent, the average value was 11.69 per cent. The cystine values for normal finger nails reported by Klauder and Brown (18) are range 10.9 to 13.5 per cent average 12.0 per cent. By a modification of Sullivan's method we obtained nail-cystine values slightly lower in some normal subjects than did Sullivan. The majority of all arthritics studied had normal content of cystine in their nails using our normal values or Sullivan's as the criterion.

Examination of Table VII shows that several of the arthritics whose sulfur metabolism was studied had low finger nail cystine values. Their basal sulfur excretion however was no different from that of subjects with normal nail cystine or controls (see Table I). Furthermore, the sulfur excretion during and after the administration of colloidal sulfur and sulfur-containing salts was no different in those subjects with low cystine content of finger nails (Tables II through V). Repeated examinations in all subjects showed surprisingly constant cystine values (whether the initial content was low or high) and in no case was there a significant increase in the cystine content of the nails after the administration of large amounts of sulfur even in those with initially low cystine content.

These data lead to the conclusion that *No significant increase occurs in the content of cystine in finger nails of persons treated with large amounts of colloidal sulfur or sulfur-containing salts. This is further evidence that sulfur administered in this way does not prevent or correct any deficiency of sulfur*

DISCUSSION

One of the most interesting and consistent results of our investigation was that, during the pe-

TABLE VII
The cystine content of the finger-nails of subjects of metabolism study

Subject	Sulfur administered	Nails obtained	Cystine
	<i>mgm</i>		<i>per cent</i>
Normals			
W A	November 11, 1938 to December 2, 1938 1503	November 5, 1938 December 6, 1938 February 24, 1939 April 5, 1939	11.32 11.40 11.36 11.34
D Mc	March 9, 1939 to March 30, 1939 1616	March 7, 1939 April 13, 1939 May 17, 1939 July 31, 1939	9.81 9.78 9.79 9.82
H M	October 24, 1938 to October 26, 1938 260	October 4, 1938 November 21, 1938 December 3, 1938	9.81 9.78 9.73
E W	April 24, 1939 to May 12, 1939 830	April 21, 1939 May 16, 1939 May 23, 1939	11.30 11.33 11.37
Initial nail cystine range 9.81 to 11.32 per cent			Average 10.56
Rheumatoid arthritis			
C B	October 21, 1938 to December 1, 1938 2146	October 21, 1938 December 12, 1938 January 12, 1939 March 1, 1939	10.32 10.28 10.33 10.31
O J	December 27, 1938 to January 28, 1939 2146	December 24, 1938 February 4, 1939 February 27, 1939 April 1, 1939 May 5, 1939 June 26, 1939 July 17, 1939	11.73 11.68 11.70 11.69 11.72 11.73 11.70
G Mc	February 16, 1939 to March 18, 1939 1866	February 2, 1939 February 28, 1939 May 8, 1939 June 7, 1939 July 26, 1939	9.31 9.28 9.62 9.46 9.37
S K	January 4, 1939 to February 5, 1939 2146	January 16, 1939 February 15, 1939 March 18, 1939 October 9, 1939	10.32 10.29 10.27 10.28
Initial nail cystine range 9.31 to 11.71 per cent			Average 10.47
Hypertrophic arthritis			
G V	June 19, 1939 to July 9, 1939 1430	June 13, 1939 July 16, 1939	8.79 8.73
D V	May 9, 1939 to May 24, 1939 2130	May 27, 1939 June 29, 1939 July 3, 1939 October 5, 1939	10.12 10.14 10.15 10.11
Initial nail cystine range 8.79 to 10.12 per cent			Average 9.45
Spondylitis rhizomelica			
S G	July 3, 1939 to July 21, 1939 1430	June 29, 1939 July 26, 1939 October 6, 1939	9.31 9.34 9.32
J S	April 6, 1939 to May 1, 1939 1090	April 2, 1939 May 6, 1939	10.61 10.58
Initial nail cystine range 9.31 to 10.61 per cent			Average 9.96

TABLE VIII

Cystine content of finger nails from persons whose sulfur metabolism was not studied

Subject	Sex	Diagnosis	Nail cystine per cent
W P	M	No disease	11.89
C. S.	M	No disease	11.58
W B	M	No disease	11.63
R. F.	M	No disease	11.32
M H	F	No disease	10.12
Cystine range		10.12-11.89 per cent	Average 11.31
L. S.	F	Rheumatoid arthritis	10.32
H J	M	Rheumatoid arthritis	10.32
J S	M	Rheumatoid arthritis	11.73
R J	M	Rheumatoid arthritis	10.82
A. T.	M	Rheumatoid arthritis	6.73
N P	F	Rheumatoid arthritis	9.32
G F	M	Rheumatoid arthritis	11.73
L. C.	F	Rheumatoid arthritis	10.43
E. S.	F	Rheumatoid arthritis	11.10
Cystine range		6.73-11.73 per cent	Average 10.28
C J	M	Mixed arthritis	11.01
M W	F	Mixed arthritis	11.20
			Average 11.10
N T	M	Arthropathia psoriatica	8.71
F B	F	Arthropathia psoriatica	12.12
			Average 10.41
M W	M	Spondylitis rhizomelica	10.63
R. H.	M	Spondylitis rhizomelica	9.93
			Average 10.28

nod when colloidal sulfur was injected the urinary excretion of sulfur increased by amounts greater than the amount of sulfur injected. We cannot explain this. This same finding was reported by Meyer-Bisch (19). He thought that it might have resulted from tissue destruction at the site of the injection of the oil suspension into the muscle because the nitrogen excretion also increased. In his patients fever occurred after the injections possibly because of muscle destruction or as a direct result of the medication. In either case increase in protein catabolism could account for the excessive excretion of both nitrogen and sulfur. Fever did not occur after intramuscular injection in any of our subjects. We observed the excessive excretion of sulfur with the injection of colloidal sulfur intravenously as well as intramuscularly. Also non sulfur-containing fluid injected intramuscularly caused no increase in nitrogen or sulfur excretion. In only one patient when sulfur was injected did nitrogen excretion

increase about parallel to the increase in sulfur elimination so that the N/S ratio definitely decreased. These observations would indicate that no appreciable muscle destruction occurred in our subjects.

Another consistent observation was that the increased excretion of sulfur, which occurred when the various sulfur preparations were given by injection or orally was chiefly in the inorganic sulfate fraction. Lewis (20) points out that this is the normal route of elimination of added sulfur. Recently Greengard and Woolley (21) reported that colloidal sulfur fed to rabbits and to humans was eliminated almost entirely as inorganic sulfate.

Our results indicate that if it should be desirable to give sulfur as medication there is certainly no biochemical or metabolic advantage in injecting it either intramuscularly or intravenously. Our results however, show no metabolic need for sulfur administration. It is interesting to note in this regard that Senturia (6) in studying urinary sulfur excretion found the same and that Wheelton (7) who is one of the most ardent advocates of colloidal sulfur therapy in arthritis, found no evidence of benefit from sulfur injection in his blood studies of sulfur and sulfur compounds.

The significance of low cystine content of finger nails is not clear. Not all patients with arthritis have low nail cystine, many have entirely normal values. Moreover low nail cystine is not peculiar to arthritis. It has been observed in patients chronically ill with other diseases (pellagra, tuberculosis, etc.). Malnutrition may account for the low values in all instances. Different authors have reported that after injecting colloidal sulfur into arthritics the cystine content of the nails increased when it had been low and frequently it became entirely normal. Some clinicians (1) have stated that best results from sulfur therapy occur in those patients with low cystine content of the nails. Comroe (22) recently stated that he noticed no definite correlation between improvement in the arthritis and increase in the cystine content of the nails nor could he predict on the basis of nail cystine which patients would benefit most by colloidal sulfur therapy.

There is no evidence that colloidal sulfur or inorganic sulfate introduced into the body in any way can be used for synthesis of sulfur-containing amino acids (20). The analyses of nails from

tically significant (for $P=0.05$), but certainly not negligible

As an explanation of this sex difference, it occurred to us that a goodly number of the young

TABLE III
Statistics concerning the cardiac output of healthy young men and women

Sex	Age	Num ber	Cardiac out put liters per minute per sq meter body surface		Cardiac out put cc. per minute per lb body weight		Cardiac out put cc. per minute per lb ideal weight	
			Mean	σ	Mean	σ	Mean	σ
M	20-29	27	2.06	9.5	24.2	11.7	26.4	10.4
F	20-29	30	1.75	12.0	22.8	13.5	22.4	13.4
M	30-39	31	2.04	11.3	23.6	12.3	25.0	11.1
F	30-39	18	1.87	8.0	23.2	10.1	23.2	9.9
Average			1.91	10.2	23.4	11.9	24.2	11.2

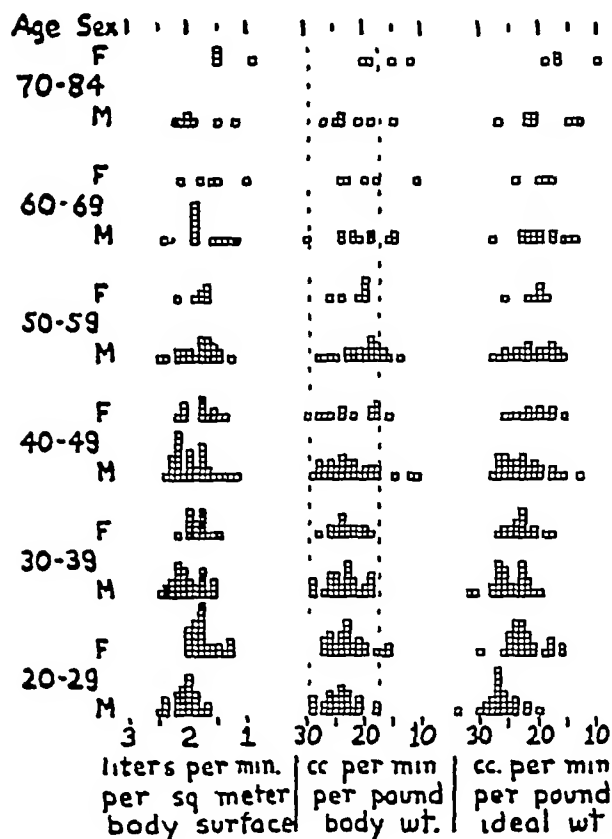


FIG. 2. FREQUENCY DIAGRAM

Cardiac output per minute of healthy persons in terms of body surface, body weight, and ideal weight, for both sexes in the adult decades. The vertical dotted lines define the normal limits which we usually employ

women were too thin, having kept their weight down for the sake of their appearance. Loss of weight does not affect the surface area proportionately so we estimated the cardiac output per minute in terms of the body weight, the results being given in Figure 2. In these data the difference between men and women is largely eliminated but the scattering is a little greater than in the preceding series.

Searching for causes of this scattering, we found that the lowest values were frequently obtained on subjects definitely overweight. Fatty tissue has an extremely small blood supply so that the weight of such subjects might be a poor index of their circulatory needs. Therefore, we estimated the cardiac output per minute in terms of "ideal weight," a figure based on height and age (4). In these data the difference between the mean values in young men and young women reappeared and the scattering was improved very little.

Inspection of the data of Figure 2 discloses a tendency for the averages to diminish with age, as was found by Lewis (7) and by Starr *et al* (5), and this is especially true when ideal weight is employed. But the differences are small, again as in Lewis' data, and they might be due to the accidental inclusion of elderly persons who were not truly normal. Therefore, realizing that further experience may cause us to change our policy, at present we prefer to use the normal limits found in the younger persons for the older ones as well. If anyone prefers to employ standards based on body surface or ideal weight, then separate standards for men and women should be employed and the normal limits may be derived from the data given in Figure 2 and Table III, the boundaries being twice the standard deviation above and below the mean. At present we prefer to use the body weight and this is discussed below.

Studies of simpler methods as indices of cardiac output

Before the cardiac output standards were compiled, we had planned to identify abnormal cases in some simpler way. We had investigated the possibility of employing the ballistic amplitude alone as an index of stroke volume and defining normal standards on this basis. After measuring the altitude of $I+J$ in mm for representa-

tive large and small complexes we made the following calculation for each case in the normal series

$$\text{Amplitude index} = \frac{(I + J + I_2 + J_2) \times \text{Pulse rate}}{\text{body weight}}$$

The results showed that normal ballistic amplitude decreased with age making it necessary to have a different standard for each decade. Also averages of the two sexes differed and the results had double the standard deviations and were scattered over twice the range of the corresponding data of cardiac output.

We also tried another index.

$$\begin{aligned} \text{Square Root of Amplitude index} \\ = \frac{\sqrt{I + J + I_2 + J_2} \times \text{Pulse rate}}{\text{body weight}} \end{aligned}$$

These results were but little better than the preceding. No shortcut has been found which gives results on normal subjects as uniform as those obtained when the cardiac output is calculated.

Limits of normality. To detect abnormal circulations we employ standards based on cardiac output per beat per pound of body weight as shown in Figure 2. The normal range has been chosen so that the chances are about 97.5 in 100 that any values falling either above or below are abnormal. From the data given in Table III we calculate a normal range of from 29 to 18 cc per minute per lb. Applying this limit to the 149 apparently healthy persons under 50 we find that 8 of them gave subnormal values. Two of these were very plump young women in the third decade who were 43 and 15 lbs. over their ideal weights, the single man in the fourth decade was extremely obese weighing 275 lbs. of the 3 men in the fifth decade 1 had once been suspected of hypothyroidism another was an arrested case of pulmonary tuberculosis who took no exercise the third was 40 lbs. over his ideal weight. Therefore one could adopt the view that this limit should not be applied to persons overweight and use the data based on ideal weight for such or one could maintain that many obese persons have abnormal circulations. As they have one symptom characteristic of the subnormal circulation (8) i.e. undue dyspnea on exertion the latter view seems more logical.

Inspection of Figure 2 shows that the great

majority of healthy persons over 50 years of age have maintained their circulations within the normal range. Of those who are subnormal possible explanations may be cited for several. One man in the sixth decade had recovered from an attack of exophthalmic goiter about 10 years before another had had myocarditis diagnosed in the army in 1917. But in most of the others no explanation was obvious although we received the impression that they were less active than their contrerers. Whether the normal limit should be lowered in the older group can hardly be decided without more experience.

Therefore a lower normal limit of 18 cc per minute per lb. body weight seems a reasonable starting point in the search for a useful division between normal and subnormal circulations.

Standards for the normal form of the ballistocardiogram

In Figures 3 and 4 are records obtained on 8 normal persons chosen to illustrate the extent of the normal variation. We have obtained records similar to these in over 600 individuals.

The normal form can be recognized at a glance. *HI* is always sharp and clearly defined. *HI* and *IJ* make acute angles with the vertical and they occur in the first half of systole. The *J* peak dominates the record.

Normal variations in systole. The prominent variation in amplitude accompanies the respiratory cycle and it is consistent with well known physiological conceptions. During inspiration the descent of the diaphragm steadily increases the pressure in the abdomen while it diminishes that in the thorax, therefore as the pressure gradient in the veins becomes greater cardiac filling increases and output increases also. During expiration the reverse takes place. This respiratory variation is always conspicuous on normal records if the breath is not held; its absence may well be of pathological significance.

The *K* wave mostly an artifact (1) varies more than the other prominent waves.

Normal variations in diastole. Waves *L* and *M* are prominent in most normal subjects (Figure 3 A) much less prominent in a few (Figure 3 B). At the end of diastole there is a ripple in some records and *H* varies with its relation to

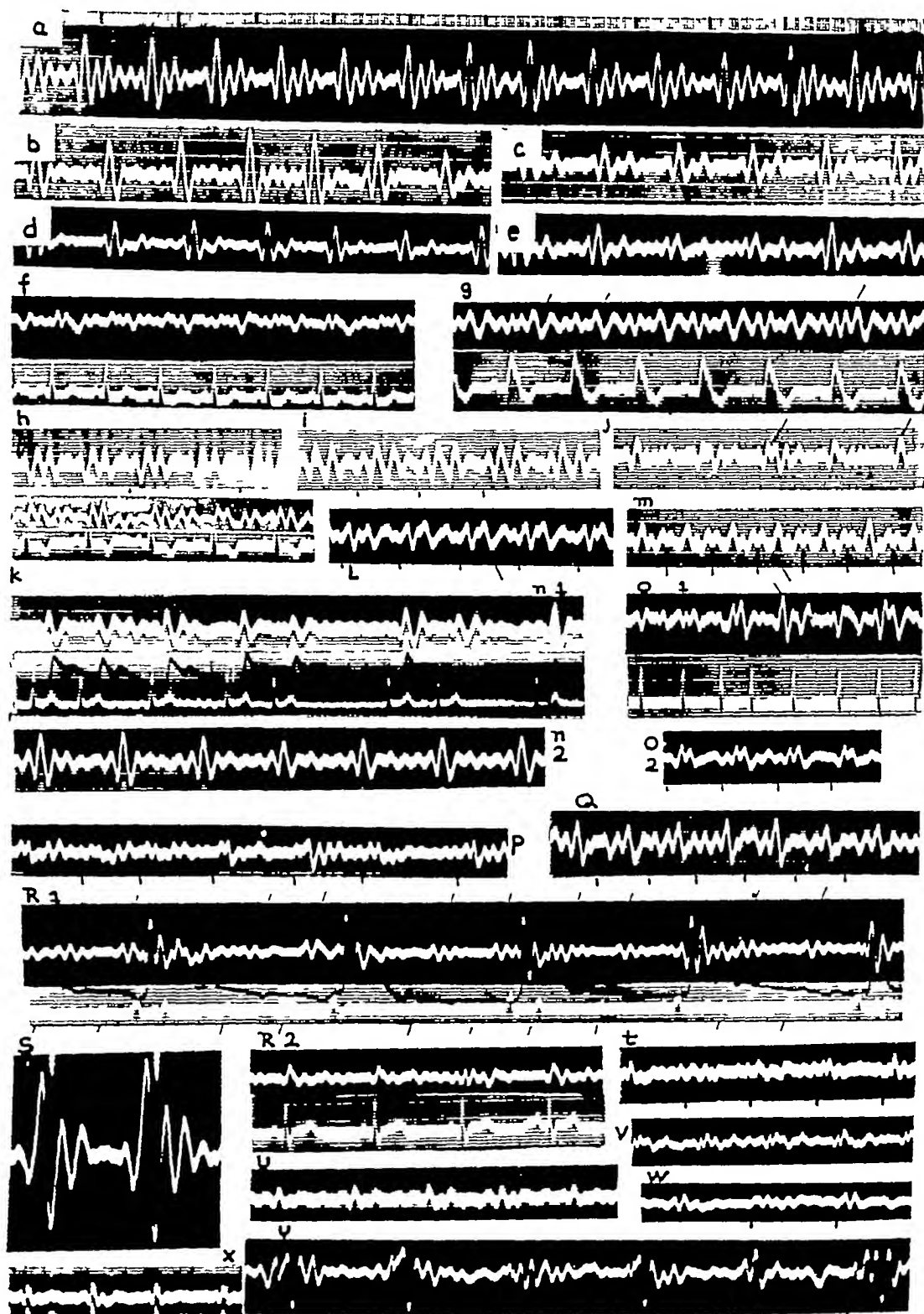


FIG 3

these vibrations. In other records the end of diastole is flat. These differences are artifacts (1). When delivered in phase the systolic impacts start the body vibrating but when the last impact is out of phase no after vibrations appear. No sig-

nificance should be attached to differences of this kind.

Description of abnormal forms

Four hundred patients have been studied in the search for abnormalities. The great majority had

FIG. 3. NORMAL AND ABNORMAL BALLISTOCARDIOGRAMS REDUCED TO $\frac{1}{2}$ ACTUAL SIZE

The time record on the top applies to all records but S. The vertical coordinates are the same for all records: the upward direction indicates a movement headward. Horizontal lines on or under records indicate the beginning of the QRS complex in a simultaneous electrocardiogram. Diagonal lines point to features of interest. Venous pressures except when indicated below were well within normal limits.

A. J. D. male age 33 height 5 ft. 7 inches weight 145 lbs BP 120/70 mm Hg diagnosis normal.

B. F. J. m. 23 5 ft. 11 152, 134/70 normal.

C. E. C. f. 44 5 ft. 124 100/65 normal.

D. C. E. m., 28 5 ft. 8 135 105/70 normal.

E. E. L. m. 64 5 ft. 10 145 130/70, normal. (A vibration in the building blurs the first part of the record.)

F. H. F. m., 46 5 ft. 146 170/110 Hypertensive cardiovascular disease. Simultaneous EKG., lead I. Note low amplitude, shallow I wave, low J prominent downstroke late in systole late downstroke type.

G. C. H. m., 56 6 ft. 170 100/65 Bundle branch block. Necropsy 2 months later showed old infarct at apex of left ventricle. Note late downstroke, shallow I in left hand part, a late M on right.

H. B. S. m. 56 5 ft. 6 170 180/130 venous pressure 20. Lues hypertension, in congestive failure necropsy 3 months later massive infarct of left ventricle. Note late M shape the first upward limb of the M is the J wave.

I. L. C. m. 36 5 ft. 8 122 120/90 Coronary occlusion 2 weeks before, recovered. Note "late M's."

J. C. M. m. 64 5 ft. 11 141 170/105 Severe angina pectoris. Note split J peaks confined to part of respiratory cycle.

K. J. R. m. 56 5 ft. 3 131 186/110 Essential hypertension, EKG suggests severe myocardial damage. Note early M's the first limb is the H wave.

L. L. B. m. 45 5 ft. 5 127 120/80 Hypernephroma. FKG left bundle branch block. Note doubling of J peak.

M. M. S. f., 23 5 ft. 10 123 102/70 Rheumatic heart disease. Mitral stenosis Class IIB. EKG shows extremely high P waves. Note flattening of I wave valley.

N. I. A. B. m. 39 5 ft. 6 148 120/80 Rheumatic heart disease. Class II-4. Mitral stenosis auricular fibrillation. EKG and brachial pulse. Note diversity of form in ballistic record largest amplitude after the pauses.

N. 2. same patient 4 days later. Blood pressure 140/85 normal sinus rhythm after quinidine. Note increased amplitude.

O. I. M. B. m., 67 5 ft. 8 197 160/110 Hypertensive cardiovascular disease auricular fibrillation. Note ballistic complexes of diverse form the large deflection in the center is probably an artifact.

O. 2. same patient 7 days later after spontaneous resumption of normal sinus rhythm. Blood pressure 160/105. Note "late M shaped tracings."

P. W. L. m. 55 5 ft. 3 160 128/85 Arterial heart disease, former coronary thrombosis auricular fibrillation. Note low amplitude and diversity of ballistic form.

Q. C. G. f. 48 5 ft. 3 188, 205/128 Arteriolonephrosclerosis. Note combination of late M and late downstroke type of ballistocardiogram.

R. I. W. P. m., 58 5 ft. 1 126 120/90 EKG and bottom of brachial pulse record. Auriculoventricular block. Note effect of isolated auricular contractions on ballistic record and variability of the ventricular complexes.

R. 2. Same subject reverted to normal sinus rhythm. Note the surprising contrast with the previous record.

S. J. F. m., 24 5 ft. 10 128 166/25 Rheumatic heart disease wide open aortic regurgitation with all peripheral signs. Cor bovinum. Note huge amplitude and normal form. In this picture the film ran more rapidly than in the others. Distance for 1 second on scale at top equals 0.67 seconds for this record. The vertical scale is identical with that of all other records in this figure.

T. P. M. f. 58, 4 ft. 8 125 210/100 Arteriolonephrosclerosis. Gall bladder disease? EKG normal. Heart size normal. Note extremely small ballistic amplitude.

U. B. H. f., 49 5 ft. 2 159 145/90 Angina pectoris myxedema. Basal metabolic rate—39 per cent. Given thyroid collapsed on street 2 months after test. Necropsy old infarct of anterior wall of left ventricle and of interventricular septum coronary sclerosis.

V. M. L. f. 48, 5 ft. 9 129 115/70 Neurocirculatory asthenia with attacks of paroxysmal tachycardia now normal sinus rhythm. Note low amplitude. J peak doubled in some complexes.

W. P. H. m. 58 5 ft. 6 152, 150/90 Diabetes mellitus. Angina pectoris for many years severe pain, possibly a fresh infarct 3 days before test recovered. Note low ballistic amplitude, form normal.

X. A. C. f., 35 5 ft. 138 160/90 Diabetes mellitus. Coronary occlusion 2 years before, angina since, died in an attack 12 months later. No necropsy. Note low amplitude.

Y. H. R. m., 46, 5 ft. 7 180 126/74 Convalescent pneumonia, bradycardia, ventricular escape. Myocardial infarct? Record obtained after giving parendrine. Note extraordinary diversity of form of the systolic complexes.

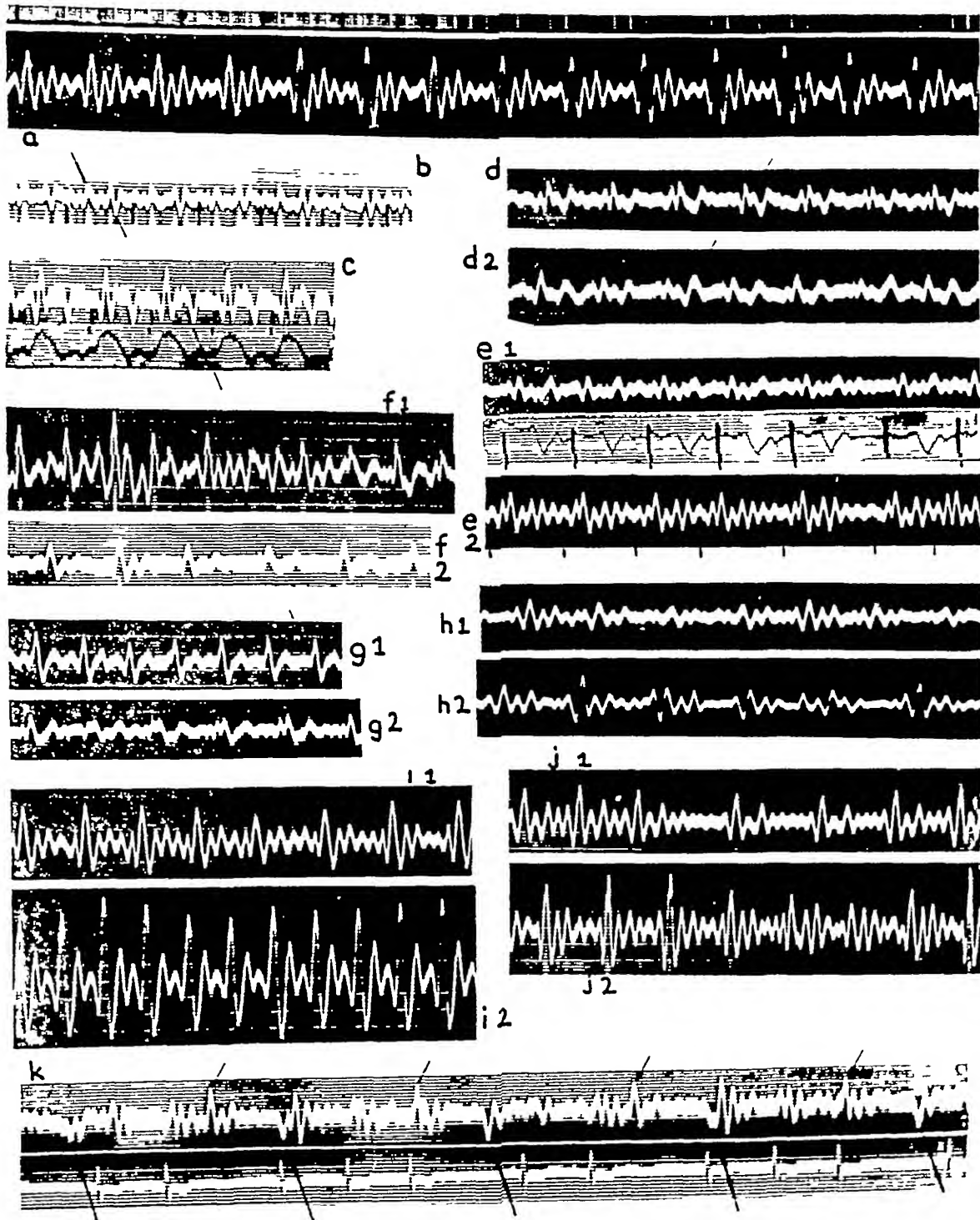


FIG. 4

records in the normal form. When abnormalities were encountered ballistocardiograms and electrocardiograms were recorded simultaneously on the same film.² In certain instances records of carotid or brachial pulses obtained by glycerine tam bours and Frank capsules were made simultaneously. In other cases the jugular venous pulse was recorded. The ballistic abnormalities encountered may be classified as follows.

The late downstroke type This is characterized by a shallow and indefinite *I* wave followed by a slow often fluctuating rise to *J* terminated by a sharp downstroke *JK* occurring late in systole and making the most prominent feature of the record. Evidence has been presented (1) that ballistocardiograms of this type are due to abnormal curves of blood velocity in the great vessels the maximum expulsion velocity not being attained until late in systole. Changes of this type have been produced experimentally in dogs by asphyxia (1).

² We are indebted to Dr. C. C. Wolfert of the Robt. Netter Foundation for the loan of an electrocardiograph for this purpose.

FIG 4 NORMAL AND ABNORMAL BALLISTOCARDIOGRAMS
1/2 ACTUAL SIZE

The time record at the top applies to all. Vertical black lines on or below the records indicate the position of the beginning of the QRS complex in a simultaneous electrocardiogram. Diagonal lines point to important features of the records. Venous pressures were normal except when given below.

A. A. R. age 40 height 5 ft. 9 inches, weight 145 lbs. B.P. 130/75 diagnosis normal.

B. L. W. 63 5 ft. 9 132, 100/70 Arteriosclerotic heart disease. Right bundle branch conduction defect. Note diastolic impacts greater than systolic.

C. M. G. 49 5 ft. 6 131 100/75 Venous pressure 15 cm. H.O. Rheumatic heart disease, mitral stenosis, tricuspid regurgitation with pulsating liver. Record with jugular pulse. Note constant diastolic downward wave in a record otherwise normal.

D. J. M. v.B. 35 5 ft. 2 165 198/130 Essential hypertension. Note notch on *jk* at certain phases of respiration.

D. 2. Same, one week later on thiocyanate. Blood pressure 130/90. Diastolic wave much more prominent.

E. 1. K. R. 37 5 ft. 11 144 105/80. Venous pressure 22. Constrictive pericarditis auricular fibrillation ascites record with jugular pulse. Note low systolic amplitude with well marked diastolic waves.

E. 2. Same 2 months post operative. Blood pressure 105/65 Venous pressure 10 Patient greatly improved

Figure 3 *F* and *G* shows examples of this type of abnormality in patients. We have encountered it in 12 subjects. Of these 7 had hypertension with marked cardiac enlargement 1 dying within a year. Another patient (Figure 3 *G*) had suffered from a coronary occlusion 2 years before which was demonstrated at necropsy 2 months later. Another patient (Figure 4 *G1*) a diabetic, showed the abnormality only when she was in severe acidosis. In contrast to these obviously sick people, the same abnormality was found in a young woman with atypical angina attacks who gave no objective evidence of heart disease and again in a man of 52 who considered himself healthy but admitted marked dyspnea on exertion. The experience at hand suggests that this abnormality indicates serious myocardial dysfunction.

The late M shaped type In this type of record the first peak of the *M* corresponds to the *J* wave of the normal record. The form can be explained by the assumption that one side of the heart is strong the other weak, the stronger ejecting blood in a normal manner produces maximum velocity early in systole while the weaker ejecting

but still had auricular fibrillation note increase in amplitude.

F. 1. J. E. 38 5 ft. 7 1/2 163 148/70 Hyperthyroid. Basal metabolic rate +35 per cent. The patient could not keep still and her movements confuse the record, although the systolic complexes can be clearly seen.

F. 2. same patient 2 months after partial thyroidectomy. Blood pressure 130/85 Basal metabolic rate -7 per cent. A striking contrast.

G. 1. E. D. 58 5 ft. 1 120 132/74 Diabetes mellitus coming out of severe acidosis flushed and weak. Note shallow or suppressed *I* wave late downstroke type.

G. 2. same, 11 days later. Blood pressure 145/90. No acidosis now. Note marked decrease in amplitude lower complexes of "early *M*" type in part of respiratory cycle.

H. 1. T. R. 60 5 ft. 8 142 96/70. Venous pressure 5.5. After severe hemorrhage from hemorrhoids.

H. 2. same 1 hour later after transfusion of 600 cc blood. Blood pressure 100/75 Venous pressure 7.0. Note increase in amplitude.

I. 1 and 2. J. B. 22 5 ft. 10 133 Normal. Before and during inhalation of amyl nitrite.

J. 1. E. C. 24 5 ft. 11 145 114/78. Normal sinus arrhythmia.

J. 2. Same 10 minutes after 0.7 cc. adrenalin S.C. Blood pressure 146/78.

K. M. F. 64 5 ft. 4 129 160/90 Arterial heart disease, irregular heart block. The diagonal lines on the record point to the effects of the diaphragmatic impacts caused by abnormally violent breathing.

with difficulty does not produce the maximum velocity until late in systole. Thus ballistic asynchronism implies neither asynchronism of the time of the cardiac systoles nor inequality of the output from the two hearts. Thus abnormality has been produced in animal experiments by damaging one side of the heart (1).

Figure 3 *H*, *I*, *J* and *O 2*, shows typical examples of this type of abnormality in patients. We have obtained such records in 19 patients. Eleven were cases of old or recent coronary infarction, 3 were proved later at necropsy. Four were cases of hypertension without evidence of cardiac infarction, 1 came to necropsy. In 3 other cases the chief diagnoses were arteriosclerosis and mitral regurgitant heart disease and hyperthyroidism. The last case a man aged 63, had the abnormality without being acutely ill, his future course will be watched with interest. Four of the 19 cases died within a year, and experience indicates that this abnormality is of serious import.

The "early M" type. This type (Figure 3 *K*) has a superficial resemblance to the preceding but the first limb of the *M* is an elevated *H* wave, not the *J* wave. It can usually be distinguished by the absence of the conspicuous *HI* downstroke before the *M* in the "early" type. The normal *H* wave is to be attributed to movement of the heart's mass during the pre-ejection phase of cardiac contraction. Exaggerated *H* waves making "early *M*" records have been most frequently recorded from cases of hypertension. In such instances it seems probable that the heart, struggling to eject blood against the increased resistance, moved so as to give a larger impact than the normal. But we have also seen this abnormality in a few patients without hypertension. One of these was a patient with a recent coronary occlusion who subsequently recovered. With this exception none of the patients showing the abnormality were very sick and it does not seem as serious as those discussed before.

Intermediate forms. The "late *M*"-shaped curves as in Figure 3, *H* and *I*, represent the extremes of ballistic asynchronism of the two ventricles and it is to be expected that all gradations will be found. Our theoretical conceptions (1) permit the arrangement of the records in a sequence. Small degrees of asynchronism would be expected to manifest themselves as a notch or shoulder on

JK as shown in Figure 4, *D 1* or as a flattening or doubling of the *J* peak (Figure 3 *L*). Larger degrees would appear as *M*'s whose central downward limb extends further and further down (Figure 3, *J* and *O 2*), until finally, in the fully developed late *M*, the central limb crosses the baseline (Figure 3 *H* and *I*). It is to be expected that records of these kinds will indicate increasing degrees of the same pathological process.

Similarly, the late downstroke type represents the extreme condition in which the blood velocity attains its maximum very late in systole and gradations from the normal to this extreme are to be expected. If both ventricles impart to the blood a maximum velocity in mid systole, the theoretical ballistic curve is characterized by a doubling of the *I* wave and a retardation of *J*. Records of this type are rare in our experience but the flattened, slightly notched *I* and the retarded *J* found in a case of rheumatic mitral stenosis (Figure 3, *M*) deserve this interpretation. A shallow *I* wave, *HI* having an abnormally obtuse angle with the vertical, is probably the first conspicuous sign of this abnormality. Figure 4, *G 1* shows a record where the *I* wave is very inconspicuous.

Abnormal diastolic waves. Preceded by a systolic complex of normal size, the normal diastolic wave is distorted by the after-vibrations. But its presence can be clearly recognized in most normal records since it causes an increase in the after-vibrations. Exceptions to this statement, such as Figure 3, *B*, are rare.

In some pathological conditions the systolic complexes are reduced in size and the diastolic waves become the most prominent feature of the record. Such records have been obtained most frequently when the venous pressure was elevated.

These abnormal diastolic waves are of two types: a sharp complex with maximum impact feetward, as shown in Figure 4, *B* and *C* by the sharp downward deflection never seen in normal records, and a more rounded upward wave, as shown in Figure 4, *D 2* and *E 1*, which resembles the normal diastolic wave but, disproportionately large, dwarfs the reduced systolic complexes, especially during expiration.

It is to be presumed that these diastolic impacts are set up by the rush of blood filling the heart and it is of interest that differences in the manner of filling may be detected in our ballistic records.

Six of the 8 records showing diastolic abnormalities were obtained from patients who were seriously ill with advanced heart disease. In 1 patient with hypertension (Figure 4 D.2) the abnormality was not constant. The last patient, who had hypertension and diabetes did not appear to be acutely ill

Variations of form from beat to beat In most records the characteristic form is maintained in every systole. The exceptions to this rule fall into two types

In the first the changes conform to the respiratory cycle. Figure 4 J illustrates this type. *M*-shaped complexes and intermediate forms alternate with normal complexes in each respiratory cycle. Apparently when well filled this heart is capable of discharging blood in a normal manner when filling pressure is lowered the abnormality appears. This type of record has always been obtained from patients whose lives were in no immediate danger but it may well indicate the beginning of a pathological process

In the second type, the changes of form have no relation to respiration. In *auricular fibrillation* the ballistic complexes vary greatly both in amplitude and in form. Often, as in Figure 3 N.1 the changes in amplitude are plainly due to the variations in rate the heart expelling more blood after the pauses which permit greater filling. But, in addition to this, as shown in Figure 3, O 1 and P, complexes of various sizes and types may follow each other indiscriminately in *auricular fibrillation*, giving impressive evidence of the disordered cardiac action. In all other conditions in which the electrocardiograms have varied from beat to beat, the impacts have also varied from beat to beat sometimes producing most bizarre records as is shown in Figure 3 Y. Extrasystoles may also produce ballistic records which vary much in form and amplitude

The same beat-to-beat variation is found in *complete heart block*. Here the differences should be attributed in part to the confusion resulting when the auricular and ventricular impacts are superimposed, in part to variations in ventricular expulsion, depending on the unequal contributions made by the auricle towards its filling. The fact that the isolated auricular beats show so clearly on the ballistic records (Figure 3 R.1) is a tribute to the sensitivity of our apparatus. In the 4 cases

of complete block tested, auricular deflections have always appeared on the ballistic record

DISCUSSION

In attempting to ascertain the clinical utility of the ballistic records two courses were open to us. We first planned to proceed empirically, describing the characteristics of the records obtained on healthy persons recording the deviations from the normal encountered in disease, and attempting to explain them by the collateral evidence bearing on the case, such as the autopsy findings x ray appearance, after histories etc. This is the chief method by which clinical knowledge of the electrocardiogram has been advanced. But a second approach was available to us because the ballistic record unlike the electrical record is directly related to the heart's sole function the pumping of blood. Therefore we studied the relationship between the ballistic record and the cardiac output a new and difficult field in which we regard our calculations as giving, at the best, only a crude approximation of the true situation (1). Nevertheless from our mathematical solution we developed a formula for cardiac output which gave results superior to the data derived from measurements of the records when both were subjected to the empirical test correlation with results obtained by another cardiac output method (1). In short by means of the theoretical approach we were able to build a better empirical method.

The results recorded in this paper show that we have repeated this experience for by calculating cardiac output even though our method is crude, we obtained a far more compact and logical set of normal standards than a simple measurement of the records provided. Therefore as the physiological approach appears more promising than the purely empirical we plan to report our results in physiological terms, as far as possible.

Limitations of the method

Tremors Tics and gross tremors may ruin the record entirely. In a few very toxic patients with hyperthyroidism the uncontrollable trembling has rendered the record useless in many such cases there is enough tremor to distort the cardiac complexes as is shown in Figure 4, F 1. Emotional

trembling may also cause trouble, but it yields readily to reassurance and attention to the patient's comfort. Good relaxation on the part of the subject is necessary to secure the best records.

Effects of abnormal respiration If the subject's breathing is sufficiently vigorous, the impacts due to the respiratory movements are so much greater than the cardiac complexes that the latter cannot be identified. In some of these patients a satisfactory record can be obtained by having them hold their breath or at least breathe less violently for a brief period. In the worst cases sufficient reduction of breathing can usually be secured after a short period of voluntary hyperpnea.

Unfortunately, if the breath is held, the size of the ballistic complexes usually varies with the position of the diaphragm, being large if it is down, much smaller if it is up. Compression of air in the lungs, if the patient closes his glottis and performs the Valsalva experiment, also influences the ballistic amplitude profoundly. Under such circumstances, one must hesitate to draw conclusions concerning the cardiac output during natural breathing from records obtained when the breath is held or respiration voluntarily reduced. However, the form of the record has its usual significance and rough estimates of cardiac output are often possible. To study severe congestive heart failure, special difficulties must be overcome.

If abnormal breathing is not vigorous enough to destroy the record, the respiratory movements may produce characteristic impacts which appear on the record and confuse the cardiac complexes. These are shown diagrammatically in Figure 5 and as they appear on a record in Figure 4, *K*. When present they must be identified or interpretation will suffer.

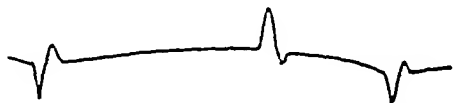


FIG 5 DIAGRAM OF THE EFFECT OF ABNORMALLY VIOLENT BREATHING ON THE BALLISTIC RECORD

Note arching of the base line and impacts when the respiratory movements change their direction.

Analysis of confused records Occasionally, with diaphragmatic impacts present, with systolic complexes varying from beat to beat, with small indefinite systolic waves and diastolic waves of equal or greater amplitude, some records may appear on first glance as confusion worse con-

founded. In such, interpretation is usually possible after comparison with records obtained when the breath is held with the diaphragm both up and down. Very occasionally additional evidence of the timing of systole, as by a simultaneous electrocardiogram, or an arterial pulse record, may be necessary to interpret the findings. These confused records have been found only in cases of heart disease of the most serious type and so we always regard them as of serious import to the patient.

Limitations in estimating cardiac output The formulae given on page 438 apply only to records in the normal form. In such cases there is ample evidence that they have the rough accuracy characteristic of the other cardiac output methods. For the abnormal ballistic forms other formulae must be employed (1) and the evidence for their accuracy is still meager. Therefore, when the form is abnormal, we seldom attempt to express the cardiac output in quantitative terms. But recalling that, in the extremes of abnormality, the cardiac output may be about 35 per cent larger than the amplitude suggests (1), we do not hesitate to express an opinion on its size in most cases. Often the amplitude is so low that a glance reveals that the cardiac output was abnormal.

The cardiac output formulae contain an estimate of the internal cross section of the aorta from the subject's age and body surface, and errors in this estimate must be expected in certain pathological conditions. Such an error makes a smaller difference than one might suppose. If the aorta or the pulmonary artery actually had an internal section area which was twice that calculated, the estimated cardiac output would be only about 17 per cent too large. Abnormal aortic dilatation can be detected by x-ray examination. If the subject's weight is abnormal, ideal weight should be used in this estimation.

The cardiac output formulae also contain an estimate of the duration of ejection from the pulse rate and this holds only for normal rhythm. It would be easy to derive a formula for cardiac output in which the duration of systole was estimated in a different way, as from the electrocardiogram. The cardiac output is a function of a root of the duration of ejection, so the effect of errors is smaller than might be supposed.

When blood regurgitates through the valves, the cardiac output estimated by the ballistocardiogram

is the whole systolic output, not that part estimated by the gas methods which contributes to the circulation. If the amount of the circulation is to be estimated, this is a disadvantage, if one is concerned with the heart's work, it is an advantage over the gas methods. Evidence has been produced that the relation of the heart's work to its size is of fundamental importance in evaluating myocardial function (5).

Relation to results obtained by other cardiac output methods

On comparing our normal standards with those obtained by other cardiac output methods it must be remembered that our results were obtained under slightly different conditions. The average resting cardiac output per sq meter of body surface for our healthy subjects of both sexes from 20 to 39 years of age was 1.91 liters. For the men only it was 2.05 liters. This compares with an average value of 2.21 liters found in young men by Grollman (6) and 2.23 liters found by the ethyl iodide method in a group of healthy persons from 20 to 39 years of age. In men from 40 to 89 years studied by Lewis (7) by the acetylene method the corresponding figure by decades varied only from 2.38 to 2.22 liters. Figure 2 shows that the mean values obtained from the ballistocardiograms tend to be a little lower than these although they are not significantly different. This is not surprising. In our method the subject lies relaxed on the table. In all the other methods he must breathe through respiratory apparatus and in some procedures, perform respiratory gymnastics. Such distractions may well prevent the degree of relaxation possible when our method is employed.

The scattering of our data about the means is less than that of Grollman's 50 medical students between 20 and 30 years of age (6) somewhat less in most decades than that found by Lewis (7) and considerably less than that found in healthy persons of varying ages by ethyl iodide (5).

When cardiac output is estimated from the ballistocardiogram, experiments on the effect of meals, mild exercise and drugs yield results essentially similar to those obtained by other cardiac output methods (9). Also in the commoner types of disease, our method yields results consistent with the older methods. This consistency

increases our confidence in the ballistic method. By its means we have discovered no generalizations concerning cardiac output which could not have been anticipated from earlier work, but to have the information available on large numbers of patients opens new fields of investigation.

Relation to results obtained by other methods of detecting cardiac abnormalities

As a general rule, when the ballistic form is abnormal, either the physical examination, the orthodiagram, the electrocardiogram, or all of them, demonstrate gross abnormalities also. The discovery of a major change in ballistic form, when all the routine clinical tests were negative, has been very rare. We can cite only 4 cases. Minor ballistic changes, such as flattening of peaks and notching of waves occurring only during expiration, have appeared a little more frequently when other tests are negative. On the other hand, normal ballistic form and amplitude in the presence of gross cardiac abnormalities are common findings giving impressive evidence of the ability of the heart muscle to compensate for its disabilities. Abnormally low ballistic amplitude, indicating a subnormal cardiac output is often found when no cardiac disease can be demonstrated.

Utility of the ballistocardiogram

Prolonged experience will be necessary before the final value of the ballistocardiogram can be fully assessed. But three promising fields of usefulness can be pointed out.

The detection of circulatory abnormalities. Patients with subnormal circulations have proved to be common in medical wards. Many have organic heart disease but a large proportion give no evidence of cardiac abnormality (8). The distinction between disease of the heart and disease of the circulation, justified by physiological reasoning and clinical experiment (5), is now possible routinely. This part of our work will be reported elsewhere.

Changes in single individuals. When the ballistocardiogram is used to assess changes occurring in one individual most of its uncertainties disappear. Figure 4 shows changes in the circulation induced in normal persons by amyl nitrite and adrenalin, in a case of hyperthyroidism by partial thyroidectomy in a case of hypertension by thio-

cyanate, in a case of constrictive pericarditis by pericardiectomy, in a case of hemorrhage by transfusion, and in a case of diabetic acidosis by recovery from the acidosis. Figure 3 illustrates the effect of changes of cardiac rhythm.

The ballistocardiogram provides an easy means of following the course of circulatory and cardiac disease and assessing the influence of treatment upon it. We believe it will be of real value in the investigation of such problems.

The subdivision of existing groups. After acute coronary infarction the ballistocardiograms of some patients give evidence of weakness of one side of the heart, in other cases both sides are equally affected. In some of these patients the cardiac output remains within the normal range, in others it is greatly diminished. After recovery from the acute episode some patients give normal records, others continue to show alarming abnormalities.

Some cases with bundle branch block show weakness of one side of the heart as one would expect, others give perfectly normal ballistic records.

Some cases of hypertension maintain their circulations within the normal range, usually by means of cardiac hypertrophy, in others the circulation is found to be diminished so that the hypertension is maintained without more cardiac work than normal. Some of these cases exhibit ballistic asynchronism as if the left ventricle was showing the first signs of being unequal to the strain, others carry a high hypertension without a trace of this abnormality.

Other groups such as rheumatic heart disease, hyperthyroidism, etc., can be similarly subdivided.

It seems reasonable to expect that information of this kind will increase our understanding of such cases and that this additional insight may lead to improved methods of caring for them.

SUMMARY AND CONCLUSIONS

Ballistocardiograms, i.e. records of the heart's recoil and the blood's impacts, have been obtained on 300 normal persons and over 400 patients. This method requires nothing of the subject save that he lie on the table. No special training is needed by the operator and the time required is about the same as for an electrocardiogram.

The *amplitude* of the ballistocardiogram is related to the cardiac output. Normal standards

for cardiac output have been defined by tests on 200 healthy persons from 20 to 84 years of age.

The *form* of the ballistocardiogram is determined by the changes of systolic blood velocity in the great vessels. The normal form has been defined. The common abnormalities have been described, their physiological interpretations set forth, and their clinical significance discussed.

The ballistocardiograph makes possible the routine estimation of the amount of the circulation over most, but not all, of the clinical field. It also provides evidence concerning cardiac health or disease of a type not obtainable by other methods.

This method seems particularly adapted to study the course of diseases of the heart and circulation in single individuals, and to assess the influence of therapeutic agents in such conditions.

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INCREASED UROBILINOGEN EXCRETION AND ACUTE HEMOLYTIC ANEMIA IN PATIENTS TREATED WITH SULFAPYRIDINE¹

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The occurrence of acute hemolytic anemia in patients receiving certain sulfonamide compounds (1) has been the subject of numerous reports. The mechanism responsible for the development of the anemia is unknown. However it has been shown by Brownlee (2), Rimington (3) and Rimington and Hemmings (4) that many of the sulfonamide drugs derange the metabolism of pigments associated with blood formation and destruction. Rimington has emphasized the fact that many of these compounds are capable of being oxidized to hydroxylamine and suggests that this oxidation product may be responsible for the breakdown of red blood cells which occasionally follows the administration of the sulfonamide group of chemicals.

During the course of administration of sulfapyridine to patients with pneumonia, acute hemolytic anemia was observed and, consequently, a study of the incidence of hemolysis following administration of the drug was undertaken.

It has been shown by a number of investigators that the estimation of the total urinary and fecal excretion of urobilinogen may be used in human beings as an index of hemolysis. By this technique increases in the rate of hemolysis may be observed which might escape detection if routine clinical procedures only are used. It should be emphasized however that the total urinary and fecal excretion of urobilinogen must be determined if an index of the rate of blood destruction is to be obtained. Estimation of urinary urobilinogen alone does not yield this information since the greater portion of urobilinogen is normally excreted in the feces. Elevation of urinary urobilinogen values represents only the increased amount of pigment diverted from the feces to the urine, such as may occur in the presence of hepatic insufficiency or during very rapid hemolysis.

The content of urobilinogen in the feces and urine varies considerably in normal patients. Watson has stated that occasionally a normal individual may excrete as much as 250 mgm. of urobilinogen per day in the stools. Values as high as this were not encountered in the study of 26 normal individuals made in this laboratory. Total stool collections were made for 3- or 9-day periods and the urobilinogen output was found to vary from 75 mgm. to 150 mgm. a day. The urobilinogen excretion in the urine varied from 0.0 mgm to less than 2 mgm a day. These figures are in close agreement with those of Watson.

The present study deals primarily with the total urinary and fecal excretion of urobilinogen by 26 patients with pneumonia of whom 20 were treated with sulfapyridine. The remaining 6 patients did not receive this drug.

METHODS

Estimation of urobilinogen in stools and urine. The Watson Terwen method (5) was used for the estimation of urinary and fecal urobilinogen. Stools and urine were collected over 3-day periods and kept in the icebox. Determination of the urobilinogen content was performed on the day following each 3-day period. Most of the patients were given milk of magnesia to ensure a daily defecation and occasionally tap water enemas were used. Diarrhea was not present in any instance.

Hematological studies. These studies were made on oxalated venous blood except in the case of stained films where capillary blood was used. Hemoglobin was estimated by the Sahli method. Red cell volumes were measured in Wintrobe tubes. The reticulocytes were counted in preparations stained supravitaly with brilliant cresyl blue and counterstained with Wright's stain.

Liver function tests. Liver function studies were made in several patients by means of the bilirubin retention test of Harrop and Barron (6) and the sodium benzoate excretion test described by Quick (7).

Urinary studies. In addition to frequent routine urinalyses in a number of instances determinations of kidney function were made by the urea clearance test of Møller, MacIntosh and Van Slyke (8).

Sulfapyridine determinations. The sulfapyridine levels in blood and urine were estimated by a modification of

¹ Given at the Thirty First Annual Meeting of the American Society for Clinical Investigation, Atlantic City May 1 1939.

TABLE I
Excretion of urobilinogen in 26 patients with pneumonia, 20 of whom received sulfapyridine

Case number	Age	Sex	Etiological agent	Sulfapyridine			Urobilinogen			Comment	
				Total dosage	Duration of treatment	Blood level during therapy		Highest excretion per day			Duration of increased excretion
						High est	Average	Feces	Urine		
				grams	days	mgm per cent	mgm	mgm	days		
GROUP I											
1	63	M	Pneumococcus Type XXV					127	0.7		
2	24	F	Undetermined					64	0.61		
3	39	F	Undetermined					62	2.2		
4	22	F	Undetermined					166	3.3		
5	29	M	Undetermined					134	0.7		
6	20	M	Pneumococcus Type VIII					70	1.07		
GROUP II											
7	44	F	Pneumococcus Type I	17.0	3	11.1	9.3	80	1.3	Coarctation of aorta Bronchiectasis Diabetes mellitus, empyema H influenzae	
8	57	M	B. friedländerii	27.0	7	10.4	6.7	206	1.6		
9	58	M	H. influenzae	18.0	4	2.2	1.7	208	3.6		
10	5	M	Pneumococcus Type I	11.5	5	7.1	6.7	85	0.75	Pulmonary tuberculosis Auricular fibrillation, acute glossitis Received Type III rabbit serum Chronic alcoholism	
11	57	M	Pneumococcus Type III	22.0	6			174	1.1		
12	66	F	Pneumococcus Type III	4.5	2			70	0.6		
13	75	M	Pneumococcus Type III	13.0	4	8.2	6.5	157	1.4	Received Type VI rabbit serum Received Type III rabbit serum	
14	52	F	Pneumococcus Type IV	22.5	5	4.9	3.4	94	2.4		
15	72	F	Pneumococcus Type VII	10.0	3	4.3	4.3	83	1.1		
16	73	F	Pneumococcus Type VI	8.0	4	4.2	4.1	120	1.0		
17	65	F	Pneumococcus Type III	14.0	4	4.6	3.1	127	1.3		
18	15	M	Pneumococcus Type VII	18.5	4	3.1	2.7	56	1.1		
GROUP III											
19	52	M	Pneumococcus Type III	78.0	10	19.6	11.0	891	4.0	14	Acute hemolytic anemia, received Type III rabbit serum, alcoholism
20	36	M	Pneumococcus Type II	81.0	11	7.7	5.8	561	0.99	10	Acute hemolytic anemia
21	59	F	Pneumococcus Type VI	18.0	4	4.5	3.0	272	0.42	3	
22	32	M	Undetermined	19.5	4	6.7	4.9	585	11.5	20	Empyema, died 2 months later of brain abscesses Acute glossitis
23	41	M	Non hemolytic streptococcus	12.0*	7			385	6.2	16	
24	52	F	Pneumococcus Type IX	24.0	8	3.9	3.7	452	1.6	7	
25	18	M	Pneumococcus Type I	20.0	5	7.3	4.6	418	2.7	6	Received Type III rabbit serum Acute azotemia, acute hemolytic anemia
26	26	F	Pneumococcus Type III	25.0	4	12.6	9.5	400	0.5	4	

* Received 10.0 grams of sulfanilamide in addition

Group I = Patients who did not receive sulfapyridine

Group II = Patients receiving sulfapyridine in whom excretion of urobilinogen was not increased

Group III = Patients receiving sulfapyridine in whom excretion of urobilinogen was increased

the method used by Marshall and Litchfield (9) for the determination of sulfanilamide.

The patients on whom the studies were made were admitted to the hospital with a diagnosis of pneumonia. The diagnosis was confirmed in each case by physical and roentgenological examination,

combined with careful studies of the sputum, blood, and exudates in order to determine the nature of the etiological agent.

Of the 26 patients, 18 suffered from pneumococcal pneumonia, and in 3 the disease was due respectively to *B. friedländerii*, a non-hemolytic streptococcus, and *Hemophilus influenzae*.

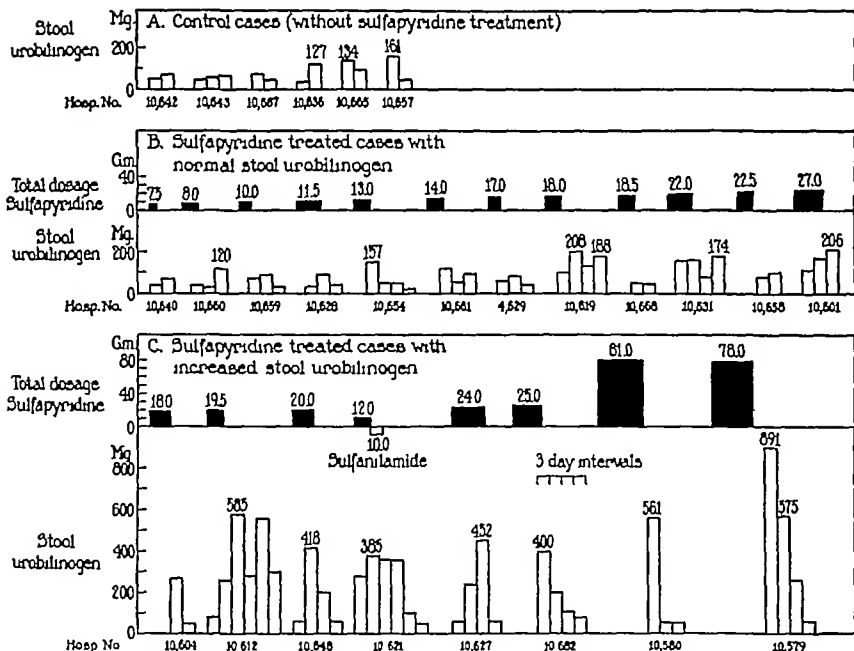


FIG. 1 EXCRETION OF UROBILINOGEN IN 26 PATIENTS WITH PNEUMONIA, 20 OF WHOM RECEIVED SULFAPYRIDINE

In 5 patients the etiological agent was not ascertained. Bacteriological examination did not reveal any microorganism which was considered of etiological importance in this group and the serial passages of throat washings and pleural exudates in mice and ferrets did not produce disease.²

Selection of patients was not made except in the case of the first 2 individuals both of whom developed acute hemolytic anemia almost simultaneously at the beginning of the study.

Sulfapyridine was administered by mouth only. The dose varied from case to case depending upon the blood level of the drug and the duration of acute signs and symptoms of pneumonia.

The patients may be divided conveniently into

three groups as indicated in Table I. The data are represented graphically in Figure 1.

Group I Patients who did not receive sulfapyridine. None of the patients in Group I showed an excretion of urobilinogen in the stools above 166 mgm per day. The highest urinary excretion was 3.3 mgm. Despite the presence of severe infection in 3 patients the excretion of urobilinogen in the feces was 70 mgm or less per day. In 4 of the 6 patients the etiology of the acute respiratory disease was not determined despite careful study. The other 2 had pneumococcal pneumonia.

Group II Patients receiving sulfapyridine in whom excretion of urobilinogen was not increased. Ten of the 12 patients in Group II had pneumococcal pneumonia. In the remaining 2 the etiological agent was *B. friedländeri* and *H. influenzae* respectively. The highest daily excretion

² These studies were performed by Dr. Frank L. Horsfall Jr., of the International Health Division of the Rockefeller Foundation.

of urobilinogen in the stools varied from 70 mgm to 208 mgm, the highest urinary excretion varied between 0.6 mgm and 3.6 mgm. The total dosage of sulfapyridine varied between 4.5 and 27.0 grams, the average blood levels of the free drug between 1.7 mgm per cent and 9.3 mgm per cent. Two patients received unconcentrated Type III antipneumococcal rabbit serum, and 1 concentrated Type VI antipneumococcal rabbit serum. In 6 patients other disorders complicated the pneumonic process.

Group III *Patients receiving sulfapyridine in whom excretion of urobilinogen was increased.* Of the 8 patients included in this group, 6 suffered from pneumococcal pneumonia. In 1 patient the etiological agent was not determined and in 1 pneumonia and empyema were due to a strain of non-hemolytic streptococcus. In the latter patient death occurred 2 months after the present studies were completed and was due to multiple brain abscesses which developed at a time when convalescence from the primary disease process was well established. Non-hemolytic streptococci were recovered from the abscesses.

The highest daily excretion of urobilinogen in patients' feces in Group III varied from 272 mgm to 891 mgm, these levels are well above those established as normal. The duration of increased urobilinogen output was from 3 to 20 days. In 4 patients the increased excretion lasted for 10, 14, 16, and 20 days, respectively. The highest urinary urobilinogen excretion varied between 0.42 mgm and 11.5 mgm per day. The total dosage of sulfapyridine varied between 12.0 grams and 81.0 grams, however, the patient receiving the smallest dose was given 10.0 grams of sulfanilamide in addition. The average blood levels of free sulfapyridine were between 3.0 and 11.0 mgm per cent. Two patients received unconcentrated Type III antipneumococcal rabbit serum as complementary treatment.

Three patients of this group developed acute hemolytic anemia. In 2, the total dosage of sulfapyridine was high—78.0 and 81.0 grams, respectively. In the third patient the output of urine diminished sharply the day after sulfapyridine therapy was begun. Acute azotemia occurred with the development of oliguria, and this was associated with high blood levels of sulfapyridine which were maintained for several days despite

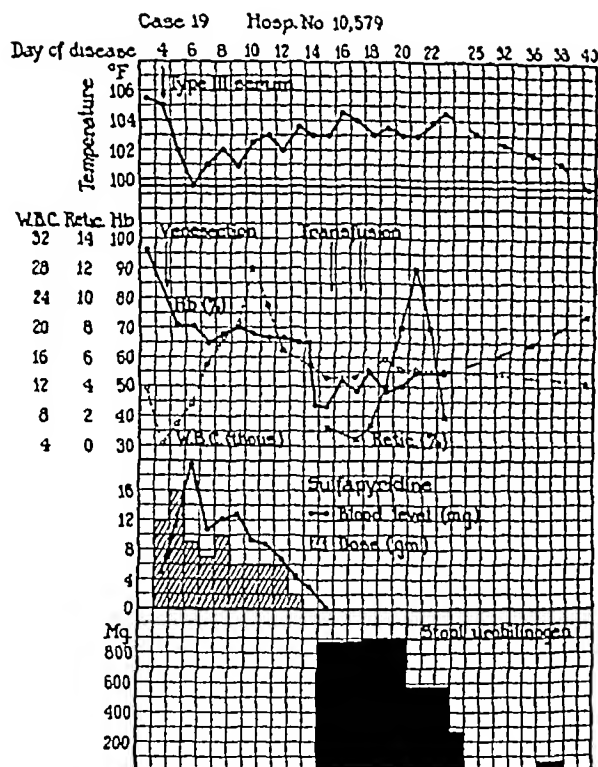


FIG. 2. ACUTE HEMOLYTIC ANEMIA IN PATIENT WITH PNEUMONIA TREATED WITH SULFAPYRIDINE

The temperature curve, blood changes, dosage and blood level of sulfapyridine, and excretion of urobilinogen in the feces are shown.

cessation of drug therapy. This patient developed a mild degree of hemorrhagic Bright's disease.

The case histories of the 3 patients who developed acute hemolytic anemia are briefly summarized.

CASE REPORTS

Case 19 (Figure 2) A white male, aged 52, was admitted to the hospital 32 hours after the acute onset of lobar pneumonia. His past history was non-contributory except for the excessive use of alcohol. On admission consolidation of the left upper lobe was present. Large numbers of Type III pneumococci were present in the sputum. Temperature was 104.6°, pulse rate 96, respiratory rate 26. The leukocyte count was 11,900, hemoglobin 96 per cent and the red blood cells numbered 5,000,000. Cultures of the blood showed no growth throughout the disease. Administration of unconcentrated Type III antipneumococcal rabbit serum was begun shortly after admission. Twenty-four hours later venesection was performed and 400 cc of blood withdrawn because of impending pulmonary edema. The hemoglobin level dropped to 70 per cent following venesection. Although Type III agglutinins were present in the patient's serum in a titer

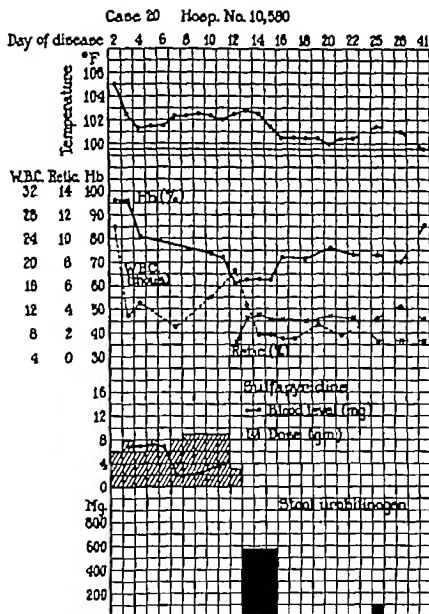


FIG. 3. ACUTE HEMOLYTIC ANEMIA IN PATIENT WITH PNEUMONIA TREATED WITH SULFAPYRIDINE

The temperature curve, blood changes dosage and blood level of sulfapyridine, and excretion of urobilinogen in the feces are shown.

of 1 256 the skin test with the Type III polysaccharide remained negative. On the second hospital day the pneumonic process spread to involve the right lower lobe. The leukocyte count declined to 4,900. Sulfapyridine administration was begun a total of 80 grams being given over the succeeding 10 days. The blood level of free sulfapyridine was 19.05 mgm. per cent 48 hours after drug therapy was begun. The dosage of sulfapyridine was reduced during the succeeding 8 days and on the day on which it was discontinued the blood sulfapyridine level was 3.4 mgm. per cent. During the course of drug therapy no extension of the pneumonic process occurred within the right lower lobe, but involvement of the entire left lower lobe took place and necessitated continuing the administration of sulfapyridine. The temperature remained irregularly elevated. The leukocyte count rose following administration of the drug 28,000 being the highest count noted. No nausea or vomiting occurred, cyanosis was minimal, and little evidence of drug toxicity was apparent.

Administration of sulfapyridine was discontinued after 10 days. The hemoglobin level had fallen from 70 per

cent to 60 per cent during the course of treatment. The following day a marked increase in pallor was observed. Blood examination showed hemoglobin 44 per cent red blood cells 1,910,000 white blood cells 14,550. The patient was transfused with 500 cc. of whole blood. Transfusion was repeated 2 days later because of continued hemolysis.

For the 3-day period during which the hemolytic process was at its height and before transfusion the daily urinary urobilinogen output averaged 4.0 mgm. while the stool urobilinogen per day for the same period averaged 891.0 mgm. Liver function was found to be within normal limits as measured by the sodium benzoate excretion and bilirubin retention tests. The urea clearance test reflected normal kidney function. There was a slight icteric tinge to the sclerae and the edge of the liver became palpable. In spite of the acute hemolytic process the reticulocyte count was below 2 per cent for the first 4 days after transfusion. A sharp rise to 12 per cent occurred during the succeeding 3 days, associated with a rise in hemoglobin to 55 per cent and of the hematocrit to 27. The daily urinary urobilinogen output averaged 1.4 mgm. fecal urobilinogen 575 mgm. per day. Three days later the urobilinogen output in the urine had fallen to 0.37 mgm. per day and in the feces to 251.0 mgm. daily indicating a marked decrease in blood destruction. Coincidentally the hemoglobin level rose to 66 per cent, the hematocrit to 29. On discharge 6 weeks later the patient's hemoglobin level was 90 per cent hematocrit 35 urobilinogen in the urine measured 0.82 mgm. daily and in the feces 63 mgm. daily.

Case 20 (Figure 3). A white male, aged 36 was admitted to the hospital 36 hours after the typical acute onset of lobar pneumonia. Temperature on admission was 104 pulse rate 120 respiratory rate 38. Moderate cyanosis was present. The red blood cells numbered 4 650 000 hemoglobin percentage was 97 and the leukocyte count was 26 000. Consolidation of the left upper lobe was present. The sputum contained large numbers of Type II pneumococci. Blood cultures were sterile throughout the illness.

Treatment with sulfapyridine was begun 2 hours after admission and was continued for 11 days a total of 81.0 grams being given. The blood level of sulfapyridine was determined frequently and found to vary considerably. The highest blood level recorded was 7.7 mgm. per cent of the free drug on the third day of treatment, and during the last 4 days the blood level varied between 2 and 4 mgm. per cent. Persistence of fever and acute signs and symptoms were associated with the development of a lung abscess in the left upper lobe, which became obvious as the surrounding acute pneumonic process resolved.

On the sixth day of drug therapy a fine macular skin rash appeared. This was confined mainly to the trunk and upper extremities and did not cause itching. The rash gradually faded and was not noted after the eleventh day.

During the course of treatment a moderate decline in hemoglobin values occurred, but on the eleventh day the patient was noticeably paler. The hemoglobin had fallen

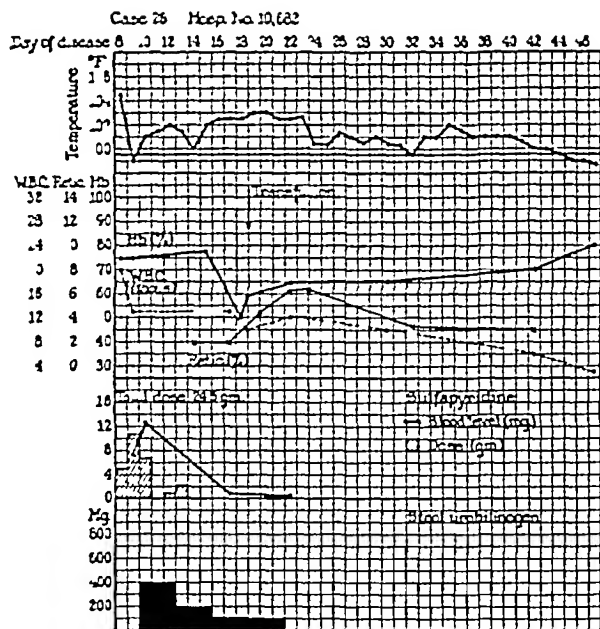


FIG. 4 ACUTE HEMOLYTIC ANEMIA IN PATIENT WITH PNEUMONIA TREATED WITH SULFAPYRIDINE

The temperature curve, blood changes, dosage and blood level of sulfapyridine, and excretion of urobilinogen in the feces are shown

to 62 per cent and the red blood count to 3,040,000, white blood cells numbered 12,800, reticulocytes 3.2 per cent, hematocrit 29. At this time the average daily excretion of urobilinogen in the stool was 560 mgm. while that in the urine averaged 0.99 mgm. Transfusion was not considered necessary. The liver was not enlarged and there was no impairment of hepatic function, as measured by sodium benzoate excretion and bilirubin retention tests. Two weeks after the administration of the drug had been discontinued the hemoglobin level had risen to 74 per cent, red blood cells numbered 3,440,000, white blood cells 10,000, reticulocytes 1.4 per cent, hematocrit 31. At this time the urobilinogen excretion in the urine was 0.40 mgm. per day, and in the feces 74.5 mgm. Convalescence was much delayed due to the lung abscess which complicated the pneumonic process. Six weeks after admission the blood examination showed hemoglobin 86 per cent, red blood cells 4,320,000, white blood cells 10,300 and hematocrit 33.5.

Case 26 (Figure 4) A white female aged 25, was admitted to the hospital on the eighth day following the onset of Type III pneumococcal pneumonia. Her past history was non-contributory. Consolidation of the right middle, right lower, and left upper pulmonary lobes was present. Temperature was 104.2°, pulse rate 132, respiratory rate 40. Blood cultures were sterile throughout the illness. Red blood cells numbered 3,790,000, hemoglobin level 74 per cent, leukocytes 21,450, urinalysis showed no abnormality, blood pressure was 112 systolic, 66 diastolic.

Twenty-five grams of sulfapyridine were given by mouth during the first 4 days following admission. Nausea and vomiting occurred and were fairly severe. The free sulfapyridine level in the blood was 126 mgm. per cent 36 hours after drug therapy was begun. This was associated with a marked diminution in the volume of urine. The patient's temperature and pulse rate fell to normal on the day following admission and agglutinins for pneumococcus Type III were demonstrable in her blood serum. The skin reaction to the Type III polysaccharide was positive at this time. Five days after discontinuing the administration of sulfapyridine, the blood level of the free drug was 10 mgm. per cent, and in the urine the level was 12.4 mgm. per cent, indicating delayed excretion. The output of urine on this day was only 300 cc. The blood urea nitrogen was 61.0 mgm. per cent. Two days later the blood urea nitrogen level had fallen to 33.6 mgm. per cent and kidney function was 62 per cent of normal, as measured by the urea clearance test.

On the eleventh hospital day, urea clearance test showed 55 per cent of normal function, 14 grams of protein were excreted in the urine in 24 hours, and large numbers of granular casts were present. Centrifuged specimens showed only 2 to 4 red cells per high power field. The blood pressure was 162/92. Edema of the face and extremities was present.

During the 4 days on which sulfapyridine was given the urobilinogen excretion in the feces averaged 400 mgm. daily, in the urine 0.2 mgm. per day. The blood examination showed practically the same findings as on admission. During the 3 days immediately following the cessation of drug therapy, the urobilinogen excretion in the feces declined to an average of 196 mgm. daily, in the urine to 0.5 mgm. By the twelfth hospital day the red blood cell count had fallen to 2,000,000, with a hemoglobin of 50 per cent, the fecal urobilinogen excretion was 132 mgm. daily. A transfusion of 500 cc. of whole uncitrated blood was given. Two days later the red blood cells numbered 3,350,000, leukocytes 20,000, and the hemoglobin 66 per cent. Urobilinogen excretion in the feces continued to fall to a level of 114 mgm. daily.

Diminution in urinary output persisted for 20 days and was followed by a period of diuresis. Four weeks after onset of the renal complication kidney function had risen to 90 per cent of normal, as measured by the urea clearance test, but the urine continued to show a trace of albumin and a few granular casts for another month. On discharge 4 months after admission, the urea clearance test showed renal function to be 96 per cent of normal, and urinalysis showed no abnormality. Blood pressure was 128/78. The red blood cell count was 4,800,000, hemoglobin 96 per cent.

Cases 19 and 20 received large doses of sulfapyridine during periods of 10 and 11 days, the total dosage being 78 and 81 grams, respectively. Administration of the drug was prolonged in both instances because of the continuation of the acute disease, in Case 19 spread of the pneumonic con-

solidation occurred and in Case 20 the acute process persisted in association with the development of a lung abscess. The blood levels of sulfapyridine are of interest in both of these patients. In Case 19 the maximum reached was 19.05 mgm of the free drug per 100 cc. of blood on the third day of treatment. During the last 5 days of treatment the level declined from 12.6 mgm per cent to 3.4 mgm. per cent. In Case 20 the highest blood level was 7.7 mgm per cent and for the last 5 days of treatment it varied between 4.0 and 2.0 mgm per cent. In Case 19 no signs of drug toxicity appeared other than acute hemolytic anemia. Despite the large dosage of sulfapyridine nausea did not occur, and there was no obvious increase in cyanosis. Case 20 suffered from severe nausea and vomiting during the whole course of drug therapy and between the sixth and eleventh days of treatment a fairly generalized macular skin rash was present.

Case 26 received much less sulfapyridine than either of the above patients—25 grams over a 4-day period. However, abnormally high levels of the free drug occurred in the blood and the excretion was delayed in association with marked oliguria.

Case 19 showed the most severe anemia of the 3 and the highest daily excretion of urobilinogen in the stools. Likewise the reticulocyte response was most marked in this case.

In all 3 patients who developed anemia the greatest depression of hemoglobin occurred about the twelfth day after the initial administration of sulfapyridine, regardless of dosage. The significance of this fact is not clear.

DISCUSSION

The widespread use of sulfapyridine in the treatment of pneumococcal pneumonia and other diseases has made a knowledge of its toxic effects important. The purpose of this communication is to present evidence for the occurrence of increased hemolysis associated with the administration of sulfapyridine. An abnormally increased excretion of urobilinogen was noted in 8 of the 20 patients who received the drug and 3 of this group of 8 developed a severe degree of hemolytic anemia.

A correlation between the increased excretion of urobilinogen and the dosage of sulfapyridine cannot be made. However, in 2 of the patients

who developed anemia a relatively high concentration of the drug in the blood was maintained for several days, in 1 by large oral dosages of sulfapyridine and in the second because of slow excretion presumably due to poor renal function.

The pneumonic process itself is apparently not responsible for increased erythrocyte destruction. The 6 patients of Group I who did not receive sulfapyridine and the 12 of Group II who were treated with the drug, excreted normal amounts of urobilinogen even though suffering from acute febrile disease. However, the 8 patients of Group III who received the drug, excreted amounts of urobilinogen well above the limits of normal. Two patients in whom urobilinogen excretion was increased during the administration of sulfapyridine later excreted normal amounts when drug therapy was discontinued even though acute febrile disease persisted.

The reason for increased hemolysis incident to the administration of sulfonamide compounds is not known. It is possible that certain patients vary in their susceptibility either to the sulfonamide compound itself or to one of the derivatives formed within the body. It is likewise possible that certain patients may convert more of the sulfonamide compound into hemolytic products than others or else fail to detoxify and eliminate these products rapidly enough to prevent increased hemolysis.

SUMMARY

The excretion of urobilinogen in feces and urine has been measured in 26 patients with pneumonia, 20 of whom received sulfapyridine.

In 18 patients the excretion of urobilinogen was within normal limits. Twelve patients were treated with sulfapyridine and 6 did not receive the drug.

Eight patients who received sulfapyridine excreted increased amounts of urobilinogen. Hemolytic anemia occurred in 3 of these.

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DISTRIBUTION OF ASCORBIC ACID IN THE BLOOD AND ITS NUTRITIONAL SIGNIFICANCE

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This paper is concerned first, with an enquiry into methods of determining ascorbic acid in whole blood and blood cells, second, with the partition of ascorbic acid between the plasma and the formed elements, and third with the significance of the apparent ascorbic acid concentration of, respectively, the whole blood, the plasma, the red cells, and the white cells and platelets as an index of vitamin C deficiency

The rapid changes in plasma ascorbic acid concentrations following the addition to or withdrawal of ascorbic acid from the diet (1, 2, 3) render a single determination of the plasma level a very limited index of vitamin C nutrition. While relatively high fasting plasma concentrations, *sc* values between 0.8 and 1.4 mgm. per 100 cc., indicate, according to present knowledge, satisfactory nutrition (4) low plasma concentrations do not provide a reliable index of either deficiency or unsaturation (1, 4, 5). Van Eekelen, Emmerie and Wolff (6), Neuweiler (7) and Heinemann (8) have presented evidence that whole blood concentrations, which include the ascorbic acid content of the blood cells as well as the plasma, reflect vitamin C nutrition to a greater extent than do plasma concentrations and actually indicate the degree of saturation or depletion. However failing the general acceptance of methods of whole blood analysis various ascorbic acid tolerance tests have been developed.

Determinations of the ascorbic acid content of whole blood, as reported in the literature, present conflicting evidence concerning the analytical procedures. Stephens and Hawley (9) and Cuttle (10) analyzed whole blood filtrates obtained after trichloroacetic acid precipitation. Kellie and Zilva (11), van Eekelen (12), Emmerie and van Eekelen (13), Borsook, Davenport, Jeffreys and Warner (14), Bessey (15), Butler and Cushman (16) and Fujita, Ebihara, and Numata (17) have found that hemolysis decreases the concentration of ascorbic acid in plasma and that precipitation

of whole blood by hemolyzing reagents such as trichloroacetic or metaphosphoric acid oxidizes ascorbic acid and invalidates results. This oxidation can be prevented by saturating the whole blood with CO or CO₂ (11, 13, 16, 17). Pijoan and Eddy (18) added a large amount of potassium cyanide to whole blood to prevent oxidation during acid precipitation. Friedman, Rubin and Kees (19), Farmer and Abt (20) and Cushman and Butler (21) have shown that the addition of such amounts of cyanide may be a source of error. Emmerie and van Eekelen (22) advocated the treatment of such whole blood filtrates with mercuric acetate to remove non-ascorbic acid reducing substances and with H₂S to recover the ascorbic acid reversibly oxidized to dehydroascorbic acid during the precipitations. The validity of this procedure has been questioned (14, 16, 23, 24, 25, 26). Heinemann (8), using this procedure, observed that the hemolysis of red cells increased the apparent ascorbic acid concentration in plasma or serum samples.

There has been little agreement concerning the distribution of ascorbic acid between plasma and red cells calculated from plasma and whole blood analyses and hematocrit determinations. Stephens and Hawley (9) found the ascorbic acid concentrations of plasma and red cells from subjects with normal white cell counts to be approximately equal. Cuttle (10), and Pijoan and Eddy (18) found the plasma concentration to exceed those of the red cells. Heinemann (8, 27) and Mirsky, Swadesh and Soskin (28) found the concentration in the plasma to be less than the concentration in the red cells. Borsook, Davenport, Jeffreys and Warner (14) observed that ascorbic acid added to whole blood *in vitro* remained in the plasma and concluded that the red cells were nearly, if not absolutely impermeable to added ascorbic acid. From *in vitro* and *in vivo* experiments Heinemann (8) concluded that added ascorbic acid passed from the plasma into the red cells.

The work reported here was undertaken in order to provide methods of analysis of whole blood and blood cells satisfactory for clinical use. It was hoped that the application of these methods might afford a more reliable index of vitamin C nutrition than is given by fasting plasma levels or by whole blood levels determined by procedures previously used in clinical investigations. The evidence for the validity of the methods we have used and data obtained by them are presented. The data show that the apparent ascorbic acid content of the whole blood and of the white cells and platelets may provide indices of vitamin C nutrition which fulfill with a fair degree of satisfaction the hope expressed above.

PART I

Examination of methods of whole blood analysis

The more commonly used indicator, 2-6 dichlorophenolindophenol, is not entirely satisfactory because it is reduced by thiosulfate, cysteine, and other substances containing the sulfhydryl group. However, under the conditions prescribed by the analytical procedures, ascorbic acid reduces the dye more rapidly than other known reducing substances (24, 29). Therefore, the rate of reduction can to some extent be used as a criterion of specificity. For this reason Meunier (30), Mindlin and Butler (31), Evelyn, Malloy and Rosen (24) and Bessey (32) have suggested the use of the photoelectric colorimeter in the determination of ascorbic acid so that the rapid reduction of the dye can be distinguished from a slow reduction. Such colorimetry, therefore, has been applied in the present study to the analysis of various filtrates.

In the photoelectric procedures the concentration of ascorbic acid in the sample solution C was calculated in the manner previously described (31), except that the procedure and calculation included, where indicated, a correction for turbidity (32)

$C = K (\log G_u - \log G_b + \log 100 - \log G_r)$, where G_u is the galvanometer reading of the unknown filtrate, G_b the reading of the dye-blank solution, and G_r is the reading of the dye-unknown filtrate solution after complete decolorization following the addition of a small crystal of ascorbic acid.

Photoelectric colorimetry has also been applied to the analysis of filtrates by a modification of Martini and Bonsignore's methylene blue method

(33). We have used this method in the analysis of whole blood for comparison with analyses in which indophenol is used and in the analysis of red blood cells where the reduction of indophenol by substances other than ascorbic acid makes that indicator unsatisfactory. Under the conditions specified in the modified procedure, methylene blue appears to be a more specific and sensitive indicator of reduction due to ascorbic acid than any other oxidation-reduction indicator. Thiosulfate, cysteine, and glutathione do not reduce the dye. The sensitivity of the procedure, as measured by change in color and galvanometer deflection, is about threefold that of 2-6 dichlorophenolindophenol. A brief outline of the method is appended to this paper. A fuller description with its application to a microprocedure will be reported later.

Using such photometry, the ascorbic acid concentrations in whole blood and plasma have been measured in filtrates obtained by the following procedures:

(1) Filtrates from plasma prepared according to the method of Mindlin and Butler (31)

(2) Filtrates from whole blood precipitated by 20 per cent trichloroacetic (9) or 10 per cent metaphosphoric acid

(3) Filtrates from plasma and whole blood prepared by the method of Emmerie and van Eekelen as described by Heinemann (8). For the photoelectric procedure the final dye-unknown filtrate, as well as the dye-blank solutions, was adjusted to pH 3.0 so that the dye did not fade as a result of the acidity (31).

(4) Filtrates from whole blood which were saturated with CO, precipitated with metaphosphoric acid and filtered in an atmosphere of CO as described in the Appendix.

Table I gives the ascorbic acid concentrations in whole blood using filtrates prepared by the methods of Stephens and Hawley (9), of Emmerie and van Eekelen (8, 22), and of CO saturation during HPO_3 precipitation. From the plasma values in the table it is seen that whole blood values by the trichloroacetic acid precipitation of Stephens and Hawley do not reflect the ascorbic acid known to be present in the plasma. In addition, known amounts of ascorbic acid added to the blood samples are not recovered after such precipitations. These results support the findings of

TABLE I

Apparent ascorbic acid concentration in whole blood found in filtrates prepared by different methods together with the plasma concentrations and data on the recovery of known amounts of ascorbic acid added to whole blood

Sample	Filtrate by method of	Mgm. ascorbic acid equivalents per 100 cc.				Per cent recovery
		Before addition of ascorbic acid		Mgm. ascorbic acid added per 100 cc.	After addition of ascorbic acid	
		Whole blood	Plasma*			
N.T.	Stephens and Hawley†	0.0	(1.1)	0.8	0.0	0
N.T.	Emmerie and van Eekelen	1.8	(1.1)			
N.T.	CO saturation plus Mindlin and Butler	1.4	(1.1)	1.0	2.4	100
A.B.	Stephens and Hawley†	0.0	(0.9)	1.3	0.0	0
A.B.	Emmerie and van Eekelen	1.3	(0.9)	0.4	1.8	100
A.B.	CO saturation plus Mindlin and Butler	0.9	(0.9)	1.0	1.9	100
E.M.	Stephens and Hawley†	0.3	(1.4)	1.3	0.7	48
E.M.	Emmerie and van Eekelen	1.5	(1.4)	0.4	2.1	110
E.M.	CO saturation plus Mindlin and Butler	1.3	(1.4)	1.0	2.3	99

* The plasma concentrations were determined by method of Mindlin and Butler (31). The values so obtained are in close agreement with values obtained from plasma filtrates by the three methods specified in the table.

† When 10 per cent metaphosphoric acid is used instead of 20 per cent trichloroacetic acid similar losses of ascorbic acid occur

other workers referred to above (11, 12, 13, 14, 15, 16, 17). There is complete recovery of added ascorbic acid when filtrates are prepared by the methods of Emmerie and van Eekelen and of CO saturation during HPO_3 precipitation. Such recovery however does not prove the validity of either method, for it does not show that the reduction in excess of that due to added ascorbic acid is a measure of ascorbic acid originally present in the blood. The data of the table show that the apparent ascorbic acid concentrations obtained by the Emmerie and van Eekelen procedure are greater than those obtained by the CO saturation procedure.

Observation of the rate of reduction in the latter two procedures provides an explanation of the difference in results. In Figure 1 the rates of reduction of the dye by a pure ascorbic acid solution by a plasma filtrate (31) and by whole blood filtrates prepared by the Emmerie and van Eekelen and the CO saturation procedures are shown. The reducing capacity expressed as mgm of ascorbic acid equivalents per 100 cc of solution plasma or blood is plotted against time in seconds. The rate of reduction of the dye by ascorbic acid varies

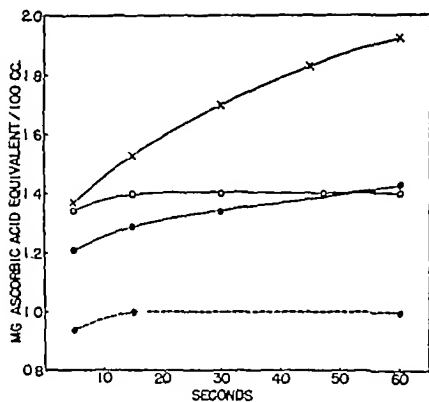


FIG. 1 RATE OF REDUCTION OF INDOPHENOL BY ASCORBIC ACID SOLUTION, PLASMA FILTRATE, AND WHOLE BLOOD FILTRATES

●---● ascorbic acid solution.
○—○ plasma filtrate.
●—● whole blood filtrate prepared by CO saturation procedure.
X—X whole blood filtrate prepared by Emmerie and van Eekelen procedure.

with the acidity of the solution (24). At pH values less than 3.0 the acidity becomes a factor in decolorizing the dye (31). Therefore, the solutions analyzed in these experiments were adjusted to pH 3. At this pH the curve for the pure ascorbic acid solution (Figure 1) reaches its height at 15 seconds and that time interval has been chosen for estimating the apparent ascorbic acid concentrations in these experiments. The plasma curve (Figure 1) is similar to that of pure ascorbic acid. Curves on plasma filtrates obtained by the Mindlin and Butler, the CO saturation and the Emmerie and van Eekelen procedures vary but little. On the other hand as shown in Figure 1 curves on filtrates of whole blood prepared by the latter two procedures continue to rise during the 60-second period, the Emmerie and van Eekelen procedure always having a steeper slope. This continued fading of the dye beyond the time interval characteristic of ascorbic acid reduction suggests reduction due to some other substance or substances. The difference in the slopes of these whole blood curves shows that the filtrate of the CO saturation procedure includes much less non-

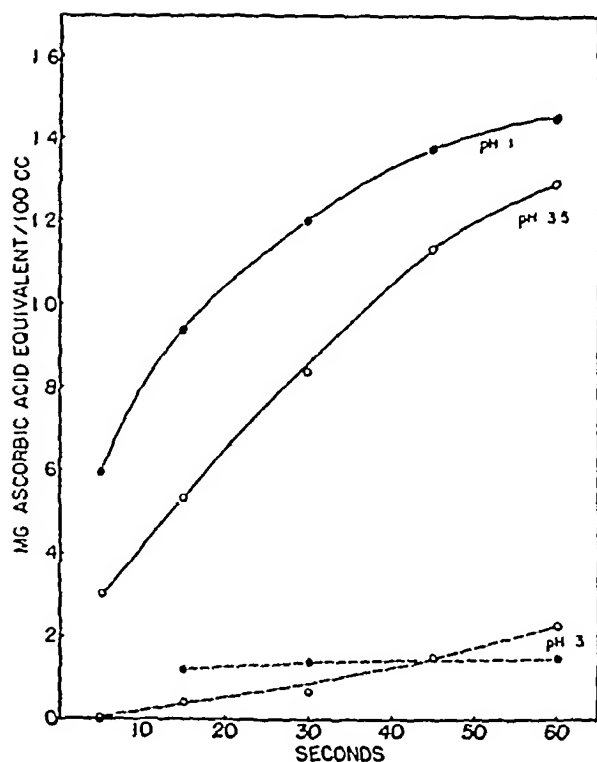


FIG. 2. RATE OF REDUCTION OF INDOPHENOL BY FILTRATES OF WHOLE BLOOD FROM SCORBUTIC PATIENTS

○-----○ } filtrates prepared by CO saturation procedure.
●-----● }
○-----○ } filtrates prepared by Emmerie and van Eekelen procedure.
●-----● }

ascorbic acid reduction than does the filtrate of the Emmerie and van Eekelen procedure

It has been suggested (23, 25, 26) that the H_2S treatment of the Emmerie and van Eekelen procedure reduces substances other than ascorbic acid, which then in turn reduce the indophenol. The data of Figure 2 give further support to such a premise. They show analyses of whole blood filtrates from four scorbutic patients whose plasmas contained no ascorbic acid. The solid line curves represent analyses of filtrates obtained by the Emmerie and van Eekelen procedure, whereas the broken line curves represent analyses of filtrates prepared by the CO saturation procedure. The difference between the slopes of the two upper curves and the slopes of the two lower curves could hardly be due to ascorbic acid but must result from substances reduced in the course of the Emmerie and van Eekelen procedure.

The data of Table II compare the apparent

TABLE II

Comparison of apparent ascorbic acid concentrations in plasma and whole blood by the indophenol and methylene blue procedures, before and after addition of known amounts of ascorbic acid

Sample		Mgm ascorbic acid equivalents per 100 cc			
		Before addition		After addition of 1 mgm ascorbic acid per 100 cc.	
		Methylene blue	Indo-phenol	Methylene blue	Indo-phenol
R M	Plasma	0.7	0.8	1.6	1.7
J M	Plasma	1.1	1.2	2.0	2.1
M C	Plasma	1.5	1.5	2.5	2.5
M C	Whole blood	1.3	1.4	2.3	2.5
M C	Whole blood	1.5	1.7	2.4	2.5
N T	Whole blood	1.5	1.4	2.6	2.4

ascorbic acid concentrations of CO saturated whole blood determined by the indophenol and the methylene blue procedures. Both procedures give values that are in close agreement. Because the procedures give complete recovery of added ascorbic acid, it seems likely that the apparent ascorbic acid values obtained include the true ascorbic acid and are not too low. Available evidence indicates that the amount of dehydroascorbic acid in whole blood is not significant (14, 23). The use of methylene blue with filtrates of whole blood obtained by the Emmerie and van Eekelen procedure is not altogether satisfactory as there is a continued fading of this dye also.

TABLE III

Apparent ascorbic acid content of whole blood in terms of the plasma, red cells and white cells-platelets, normal subjects

Sample	Volume per 100 cc blood		Mgm. ascorbic acid equivalents per 100 cc. by analysis				Calculated mgm. ascorbic acid equivalents per 100 cc. whole blood			
	Red cells	White layer	Plasma	Red cells	White layer	Whole blood	In the plasma	In the red cells	In the white layer	Total by addition
	1	2	3	4	5	6	7	8	9	10
1 A.B.	39.8	0.6	0.8	0.4*	25	0.8	0.47	0.16*	0.15	(0.8)
2 F.	42.5	0.8	1.0	0.9*	32	1.3	0.57	0.37	0.26	(1.3)
3 B.	43.1	0.4	1.1	0.6	38	1.1	0.62	0.26	0.15	1.0
4 M.C.	41.5	0.7	1.5	0.7	36	1.4	0.87	0.29	0.25	1.4
5 M.C.†	41.0	0.5	1.2	1.1	38	1.6	0.70	0.45	0.18	1.3
6 M.C.†	40.5	0.5	1.4	1.4	35	1.6	0.83	0.57	0.18	1.6
7 R.M.	51.4	0.7	0.8	0.7	34	1.1	0.39	0.26	0.24	1.0
8 R.M.‡			2.5	1.0		1.9	1.20	0.51		2.0
9 R.M.‡			1.9	1.5		1.8	0.91	0.77		1.9
10 R.M.‡	51.0		1.2	1.0		1.2	0.53	0.51		1.3

* Calculated from plasma, whole blood, and hematocrit values.

† On a diet high in ascorbic acid. See text.

‡ A saturation test following 700 mgm ascorbic acid. See text.

Though satisfactory criteria for the validity of whole blood ascorbic acid analyses are lacking the data presented demonstrate that precipitation of whole blood by the CO saturation procedure outlined here is not accompanied by loss of ascorbic acid by oxidation and that filtrates obtained by this procedure contain less interfering reducing substances than filtrates obtained by the Emmerie and van Eekelen procedure.

Therefore, the whole blood and red blood cells analyses presented in the following sections have been carried out on filtrates obtained by the CO saturation procedure.

PART II

Whole blood ascorbic acid partition between the plasma the red cells and the white cells and platelets

Table III shows the mgm. of apparent ascorbic acid per 100 cc. of plasma, of red cells, of white cells and platelets¹ and of whole blood determined by direct analyses in samples of blood from normal subjects. The plasma and white cell plus platelet analyses were made according to the procedure of Mindlin and Butler (31) the whole blood analyses by the indophenol or methylene blue procedure applied to filtrates obtained by CO saturation and metaphosphoric acid precipitation, and the red cell analyses by the methylene blue procedure applied to filtrates similarly obtained. Actually the concentrations in the white cells plus platelets were determined per 100 grams of cells. However for convenience of tabulation and without significant error the results are recorded as per 100 cc. From the concentrations in the three phases and the volumes of red cells and of white layer per 100 cc. of whole blood the mgm. of apparent ascorbic acid in the plasma the red cells, and the white cells plus platelets in 100 cc. of whole blood were calculated and are given in the table. Finally the sum of these values is given

for comparison with the whole blood content found by direct analysis

By comparing the concentrations of apparent ascorbic acid in the plasma and red cells in Tables III and IV it is seen that the ratio of plasma ascorbic acid concentration to red cell ascorbic acid concentration varies from 2.6 to 0.3. The first sample from Subject M C Table III, was taken in the mid morning after a vitamin C-free breakfast when on a routine diet without added ascorbic acid. The second and third samples were taken at similar times after one week's daily ingestion of 250 mgm and 500 mgm. respectively of ascorbic acid. The ascorbic acid concentrations of the red blood cells rose from 0.7 to 1.4 mgm per 100 cc. as the ingestion of ascorbic acid increased, while the plasma and the white cell platelet ascorbic acid concentrations showed no significant change. The first sample of blood from Subject R. M. was a fasting specimen taken immediately before the ingestion of a single dose of 700 mgm of ascorbic acid. Samples were then taken 3, 7, and 24 hours later. The plasma ascorbic acid concentration rose over the first 3 hours and fell during the next two periods. The ascorbic acid concentration of the red blood cells rose less rapidly the concentration at 7 hours being the highest value. The data of the tables thus show the importance of the nutritional state and the time interval between the ingestion of ascorbic acid and the taking of the blood sample in respect to the plasma and red blood cell ascorbic acid concentrations. They also show the slow penetration of ascorbic acid into the red cells as reported by Heinemann (8).

Taking the average white cell platelet apparent ascorbic acid concentration of 34 mgm. per 100 cc. of white layer as a normal value (Table III) and taking the average volume of white layer per 100 cc of blood as 0.6 cc. the white layer accounts for approximately 0.2 mgm of ascorbic acid per 100 cc. of blood. Indeed, the amount of ascorbic acid that the white layer contributes to several of the whole blood ascorbic acid concentrations of Table III is approximately equal to that contributed by the red blood cells. That the apparent ascorbic acid concentration of the white layer in abnormal states may be the major factor in whole blood values is shown in the following section

¹ By microscopic examination the upper pure white portion of the white layer consists of platelets with a few white cells while the lower buffy portion consists largely of white cells with some platelets and a few red cells (35 b). Analyses of these two portions, however have given within the error of the method the same reduction or apparent ascorbic acid concentration.

PART III

The apparent ascorbic acid content of the white cells and platelets

Stephens and Hawley (9) and Cuttle (10) observed a high concentration of indophenol-reducing substance in the white blood cells of human subjects. Kellie and Zilva (34) found that the white blood cells of guinea pigs had a high reducing power. This increased comparatively little after the intravenous administration of large quantities of ascorbic acid. Its concentration fell when the animals were placed on a scorbutic diet, but there was a residual reducing substance or substances which could not be identified by these authors.

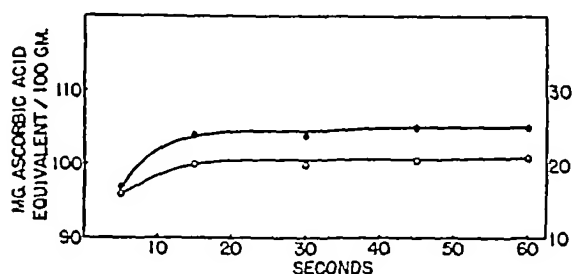


FIG 3 RATE OF REDUCTION OF INDOPHENOL BY FILTRATES FROM METAPHOSPHORIC ACID PRECIPITATION OF NORMAL AND LEUKEMIC WHITE BLOOD CELLS

○—○ leukemic cells ordinate on left
●—● normal cells ordinate on right

Figure 3 shows the time curve of reduction of indophenol by metaphosphoric acid filtrates of the white layer of centrifuged blood from normal and leukemic subjects. It will be seen that the curve corresponds to that of pure ascorbic acid in Figure 1. The reaction of the reducing substance with methylene blue also corresponds to that of ascorbic acid. Because neither plasma nor white cell-platelet filtrates give delayed reduction, such reduction by filtrates from whole blood obtained by the CO saturation procedure must be due to substances within the red cells. This conclusion is further substantiated by analyses on such filtrates from samples of red blood cells in which, as already remarked, slowly acting reducing substances make analyses with the indophenol dye unsatisfactory.

Table IV presents analyses of whole blood ascorbic acid equivalents in terms of the plasma,

TABLE IV

Apparent ascorbic acid content of whole blood in terms of the plasma, red cells, and white cells-platelets from subjects on diets deficient in vitamin C

Subject	Type of deficiency	Volume per 100 cc blood		Mgm. ascorbic acid equivalents per 100 cc. by analysis				Calculated mgm. ascorbic acid equivalents per 100 cc. whole blood			
		Red cells	White layer	Plasma	Red cells	White layer	Whole blood	In the plasma	In the red cells	In the white layer	Total by addition
C	Acute scurvy	35.0	1.0	0.0	0.0	3	0.0	0.00	0.00	0.03	0.03
	After vitamin C	35.0	1.3	0.3	0.8	23	0.8	0.20	0.21	0.59	0.70
Y	Acute scurvy	33.7	1.1	0.0	0.0	0	0.0	0.00	0.00	0.00	0.00
	After vitamin C	32.4	1.1	0.1	0.3	12	0.4	0.07	0.10	0.13	0.30
Ly	Acute scurvy	46.0	0.3	0.0	0.0	2	0.0	0.00	0.00	0.01	0.01
	After vitamin C	51.0	0.4	0.0	0.0	12	0.0	0.00	0.00	0.05	0.05
	After vitamin C	49.0	0.4	0.1	0.3	23	0.3	0.05	0.15	0.09	0.29
	After vitamin C	50.0	0.3	1.0	1.1	33	1.3	0.50	0.55	0.11	1.16
Bl	3 weeks experiment	51.0	0.9	0.2	0.3	29	0.4	0.10	0.15	0.25	0.51
	4 weeks experiment	46.0	0.6	0.1	0.1	22	0.2	0.05	0.05	0.13	0.23
Cr	3 weeks experiment	49.0	0.6	0.3	0.2	28	0.3	0.10	0.10	0.16	0.36
	4 weeks experiment	49.0	0.5	0.1	0.1	24	0.1	0.05	0.05	0.13	0.23
	6 weeks experiment	47.0	0.4	0.0	0.0	10	0.1	0.00	0.00	0.04	0.04
	12 weeks experiment	43.0	0.6	0.0	0.0	3	0.0				
G	Diet by history	0.5	0.0			21	0.3	0.00		0.11	0.11
Cer	Diet by history	37.0	1.1	0.0	0.2	12	0.2	0.00	0.07	0.13	0.20

the red cells and the white cells and platelets from subjects receiving diets deficient in vitamin C. In the untreated scorbutic patients the apparent ascorbic acid in the white cells and platelets varied from 0.0 to 3.0 mgm per cent compared to a variation in the normal individual from 25 to 38 mgm per cent (Table III). Following vitamin C therapy there was a significant rise in the reducing substance in the white layer and in the whole blood of the scorbutic patients before such a rise occurred in the plasma. The data for Subject Ly show the return of the white layer concentration to a low normal value during a period when the fasting concentration of the plasma rose to but 0.1 mgm per cent. One infant, not reported in the table, recovered from all the symptoms of acute scurvy and by a saturation test² showed fair saturation without the fasting plasma level prior to the test going above 0.2 mgm per cent.

Subjects Bl and Cr³ were on a self-prescribed deficient diet. The data show the fall in plasma concentrations to very low levels before there was an appreciable drop in the white cell-platelet concentrations. The fall in the apparent ascorbic acid content of the red cells with the drop in plasma concentration resulted in a decrease in the

² Performed by Dr R. L. Mindlin

³ The samples of blood from these two subjects were provided through the kindness and cooperation of Dr John Crandon

whole blood level independent of and earlier than the decrease in white cell platelet concentration. At the end of 12 weeks on the deficient diet the apparent ascorbic acid concentration of subject Cr's white blood cells plus platelets had fallen to 3 mgm per 100 grams of white layer. Though the subject felt below par, no definite signs of scurvy had appeared.

Infants G and Cer had no ascorbic acid in the plasma and 21 and 12 mgm per cent, respectively in the white layer of centrifuged blood. Each had a dietary history suggesting vitamin C deficiency but, as might be expected from the preceding data, neither presented any roentgenological or clinical signs of scurvy.

These findings indicate that the whole blood and the white layer of centrifuged blood of subjects whose vitamin C nutrition is relatively poor may contain measurable amounts of apparent ascorbic acid after the plasma level has become zero. *Therefore, the apparent ascorbic acid content of the whole blood or of the white blood cells plus platelets of individuals not suffering from infection or leukemia provides an index of vitamin C deficiency that extends beyond the limits defined by plasma values.* Because the white cell platelet concentration is less dependent upon fluctuations in plasma concentration than is the whole blood content the former appears to be the best index of physiologically significant deficiency. On the other hand it is clear from the data of Tables III and IV that white cell platelet analyses will not provide maximal information concerning the saturation of subjects whose vitamin C nutrition is relatively good because high white cell platelet concentrations are found early in the recovery from and late in the development of deficiency and therefore are constantly high in relatively well nourished subjects. In this sense the data support Heinemann's conclusion that high concentrations in both cells and serum seem to be the phenomena in blood which really characterize saturation in the strictest sense.

Although the milligrams of ascorbic acid in the white cells and platelets per 100 cc. of whole blood are proportional in each patient with the vitamin C nutrition these values are not so clearly informative as the white cell platelet ascorbic acid concentrations. Further data from subjects with

TABLE V

Apparent ascorbic acid content of whole blood in terms of the plasma, red cells and white cells platelets from patients with leukemia

Patient	Type of anemia and therapy	Volume per 100 cc. blood		Mgm. ascorbic acid per 100 cc. by analysis			Calculated approx. ascorbic acid per 100 cc. whole blood	
		Red cells	White layer	Plasma	White layer	Whole blood	In the plasma	In the white layer
I	Lymphatic leukemia	32.8	6.5	0.3	100	7.6	0.2	6.8
St	Myelogenous leukemia	30.7	6.8	0.3	47	4.3	0.2	3.1
Ye	Myelogenous leukemia	8.0	12.0	0.3	47	4.3	0.3	6.1
HI	Lymphatic leukemia	33.0	6.0	0.1	48	2.8	0.1	2.9
He	Lymphatic + vitamin C	32.0	6.0	0.3	85	6.0	0.2	4.7
Pe	Lymphatic leukemia	14.0	8.7	0.0	32	0.0	0.2	2.6
Pe	Lymphatic + vitamin C	10.9	8.1	0.3	70	7.0	0.2	6.4
Sam	Lymphatic leukemia	30.1	3.7	0.3	90	4.0	0.1	3.3
Bm	Lymphatic + vitamin C	29.9	3.7	0.3	139	8.3	0.3	8.3

infection and shifting white cell and platelet counts should provide information on this point.

Stephens and Hawley (9), Cuttle (10), and Butler and Cushman (16) have reported high concentrations of an indophenol reducing substance in leukemic white cells. The rate of reduction of indophenol (Figure 3) and the reduction of methylene blue by the reducing substance of the white layer is similar to that of ascorbic acid. Table V presents examples of the high reducing capacity of the white layer of centrifuged blood from leukemic patients. From the plasma, white layer and whole blood values it is seen that the relation between the ascorbic acid concentrations of the plasma and the apparent ascorbic acid concentration of these leukemic cells is very different from that in normal subjects. The white cell-platelet values exceed the maximum normal values even though the plasma levels are low. It is of interest that the reducing power of the white layer of the last three patients of Table V increased with an increase in vitamin C intake. However though the relative reducing power of leukemic cells and platelets may be proportional to the vitamin C intake their absolute reducing capacity does not reflect vitamin C nutrition as do the values reported in Table IV. It is for this reason that an exception has been made of leukemic subjects when concluding that the apparent ascorbic acid concentration of white cells plus platelets is an index of deficiency. A subsequent paper will deal with the identity of the reducing substance or substances found in the blood of leukemic subjects.

SUMMARY

Errors involved in the determination of the ascorbic acid concentration of whole blood are discussed.

Procedures for the analysis of the ascorbic acid content of red blood cells, white blood cells plus platelets, and whole blood are described.

Because the reducing power of the white cells plus platelets varies from an average normal of 34 mgm of ascorbic acid equivalents per 100 grams of white layer to a level of approximately 0.0 in scorbutic subjects, we conclude that this reducing substance is ascorbic acid or some substance metabolically associated with it.

The data show that ascorbic acid passes from the plasma to the red cells and that the distribution ratio of the plasma concentration to apparent red cell concentration varies with the state of vitamin C nutrition.

They show that the apparent ascorbic acid concentrations of the white cells plus platelets and of the whole blood of individuals not suffering from infection or leukemia provide indices of vitamin C deficiency which extend beyond the limit of the index furnished by fasting plasma concentrations. They suggest that the apparent ascorbic acid concentration of the white cells plus platelets of such individuals is the best index of physiologically significant deficiency. They also indicate that the apparent ascorbic acid content of red blood cells or of whole blood is a better index of saturation, as differentiated from unsaturation or deficiency, than plasma or white cell-platelet concentrations.

The occurrence of very high concentrations of an ascorbic acid-like reducing substance in the white layer of centrifuged blood from leukemic patients is confirmed.

APPENDIX

1 CO saturation and precipitation of whole blood

Add 1 drop of caprylic alcohol to 2 cc. of whole blood and bubble CO through this for 10 minutes. While the CO is still passing through the sample, add 12 cc. of water and, after 5 minutes, 2 cc. of 32 per cent HPO_3 . Thoroughly mix by means of the bubbles of CO for 30 seconds. Then immediately deliver the precipitated blood to a filter in a glass container through which CO is passing and collect the filtrate. The precipitate on the filter paper should be bright red. Darkening of the precipitate means loss of ascorbic acid by oxidation.

2 White blood cell-platelet analysis

Twelve to 15 cc. of whole blood are collected in a flask containing ammonium and potassium oxalate as described by Wintrobe (35). Determine the hematocrit on 0.5 cc of the oxalated blood. Depending upon the hematocrit, 10 or 12 cc. of blood are transferred to a centrifuge tube constricted near the middle to a capillary 3 mm. diameter and 10 mm length in which the white cells and platelets after centrifugation will be packed above the red cells. After centrifuging an hour or more at high speed the plasma is pipetted off. By means of a capillary pipette 10 to 20 milligrams of white layer are transferred to a centrifuge tube containing 0.5 cc. of 45 per cent HPO_3 , which has been weighed with the HPO_3 , before addition of white cells. A second weighing gives the weight of white cells and platelets. The cells are pressed with a glass rod, 1 cc. of 45 per cent HPO_3 is added, and the contents are shaken thoroughly.

After centrifugation, pipette 0.8 cc. off the supernatant fluid to a micro-colorimeter cell and add 0.2 cc. indophenol-acetate solution, containing equal parts of 5 per cent 2-6 dichlorophenolindophenol and 5/3 M acetate buffer. This gives the desired pH as described by Mindlin and Butler (31). The galvanometer reading of this mixture and of a metaphosphoric acid and indophenol-acetate solution dye blank is recorded and the results are calculated as stated in the body of this paper.

3 Methylene blue procedure

To 4 cc. of filtrate from whole blood treated by the CO saturation and metaphosphoric acid precipitation procedure described above are added 4 cc. of a 0.5 mgm. per cent buffered methylene blue solution. This buffered dye solution contains equal parts of 10 mgm per cent methylene blue and the acetate buffer solution referred to (31). The resulting pH of the filtrate dye solution mixture should be about 4. A similar mixture containing no ascorbic acid is prepared for the blank dye solution. Place the colorimeter tubes containing the unknown dye and blank-dye solutions in a beaker of water and place this before a 500 watt electric light with reflectors focused on the tubes in the beaker. After a 1-minute exposure, add 2 cc. 0.5 N HCl to each tube, immediately discontinue the illumination and mix the contents of each tube. Using a filter that transmits light from approximately 600 to 700 μ , take the galvanometer reading in the photometer with the center setting adjusted to read 100 with the dye completely decolorized. Calculate the result as described, using a K value determined by analyses of known ascorbic acid solutions.

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FACTORS THAT INFLUENCE THE PASSAGE OF ASCORBIC ACID FROM SERUM TO CELLS IN HUMAN BLOOD

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Previous work has demonstrated that ascorbic acid, which has been added to defibrinated blood is taken up by the cells (1). The advantages offered by the method of Mindlin and Butler (2) have made it possible to investigate the factors which influence the passage of ascorbic acid into the cells. This paper deals with the effect of time, temperature and oxygen

METHODS

Ascorbic acid in serum was determined by the method of Mindlin and Butler (2) with the following modifications.

(1) Neither potassium oxalate nor potassium cyanide was used. An anticoagulant was not necessary because the blood was defibrinated by stirring with a glass rod. The possible erroneous influence of added KCN has been demonstrated by other observers (3 4 5) as well as in this laboratory. Furthermore, addition of oxalate, cyanide or any other salt seemed undesirable, since changes in cell volume had to be avoided.

(2) The strength of the metaphosphoric acid solution is of great significance for the reliability of the colorimetric method. With ascorbic acid a stable color develops other reducing substances cause progressive fading of the indicator Mindlin and Butler pointed out, and our own observations agree that, with solutions of pure ascorbic acid the stability of the reduction of the dye depends on the pH of the final mixture of equal volumes of buffered dye and metaphosphoric acid. The dye solution invariably gave the desired pH of 7.0 when made according to the directions given by the aforementioned authors. Metaphosphoric acid solutions, however made up by weight from three different brands, varied widely in strength and were all weaker than theory demanded. Since it is known that metaphosphoric acid is partially converted to the ortho form on standing the strength of a solution can be adjusted only by standardization. The metaphosphoric acid was, therefore, titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. In agreement with the observations of Mindlin and Butler, stable blanks were obtained when the final pH of the dye-metaphosphoric acid mixture was kept at 4.2 to 4.3. This resulted with solutions of the acid which were 0.21 to 0.22 N. The same normality was attained in

filtrates from serum when the deproteinization had been carried out with a solution of this acid 0.51 and 0.52 N.

(3) *Procedure* In most of the experiments large amounts of ascorbic acid were added to the blood. Smaller aliquots of serum were therefore necessary. In control experiments identical results were obtained on filtrates derived from 0.5 cc., 1 cc. or 2 cc. of serum, provided proportional volumes of distilled water and metaphosphoric acid were used in the precipitation. When 0.5 cc. was used the precipitate was thrown down by centrifuging to insure 1 cc. of filtrate for analysis. This was then diluted to 6 cc. with 0.21 N metaphosphoric acid. Four cc. of this diluted filtrate added to an equal volume of the buffered dye solution were used for the colorimetric reading.²

Determinations of ascorbic acid on filtrates made from ten 0.5 cc. aliquots of the same serum gave results which varied by not more than 2 per cent.

(4) *Calculation.* Repeated series of known amounts of ascorbic acid in metaphosphoric acid solution (0.21 to 0.22 N) were read in the photoelectric colorimeter. The concentrations of ascorbic acid ranged from 0.4 to 2.4 mgm. per cent. The constant k used in the equation $C = K (\log G_0 - \log G_s)$ was found to vary between 1.010 and 1.108, averaging 1.059 ± 0.049 . The stability of this constant was not studied further since in this investigation comparative and not absolute measurements of ascorbic acid were contemplated.

Ascorbic acid in whole blood was determined by the method of Emmerie and van Eekelen (6) as previously described (1). In a few instances, for purposes of comparison, ascorbic acid in serum was also measured by this method.

Blood cell volumes were measured by the hematocrit method described by Eisenman, Mackenzie and Peters (7).

EXPERIMENTAL RESULTS

Crystalline ascorbic acid was added to human blood which had been defibrinated with a glass rod. In order to avoid hemolysis and changes of cell volume, it was not introduced directly into the whole blood but was first dissolved in a portion of the serum. (It was demonstrated by innumerable controls that the cell volume was not al-

¹ This work was aided by a grant from the Markle Foundation.

² Although the Evelyn colorimeter in use in this laboratory has no 8 cc. aperture, it was found that satisfactory readings were obtained when the 10 cc. aperture was used.

tered by any of the experimental procedures employed in this investigation) The defibrinated blood was then divided into two parts. From one the serum was separated at once and analyzed for ascorbic acid. The remainder of the serum, which will be spoken of as "separated serum," and the remaining portion of whole blood were then placed in stoppered Erlenmeyer flasks and kept at the same temperature. Samples of the blood, removed at intervals, were centrifuged, and the sera thus secured were analyzed for ascorbic acid. These sera will be referred to as "true serum"—that is, serum which has been in contact with the blood cells up to the moment of analysis. Portions of the separated serum were analyzed at the same intervals. This permitted a comparison of the behavior of serum with and without contact with the blood cells.

Ten studies were conducted at refrigerator temperature (approximately 7°) under atmospheres of both air and nitrogen, with and without shak-

TABLE I
Stability of ascorbic acid in defibrinated whole blood under various conditions

Experiment number	Time of incubation minutes	Ascorbic acid in whole blood	
		Air	Nitrogen
1	0	240.6	
	195	243.7	
	285	240.6	
2	0	219.0	
	255	219.0	
	375	215.3	
	480	220.5	
3	30	47.5	
	180	47.5	
	360	46.0	
	540	42.5	
4	30	48.8	
	180	46.3	
	360	42.5	
	540	40.0	
5	30	58.3	
	540	47.2	
6	0	63.9	63.9
	255	44.4	
7	0	68.8	69.9
	255	50.0	

Experiments numbers 1 and 2 at approximately 7° C., numbers 3, 4 and 5 at 37° C., numbers 6 and 7 at 37° C., while continuously shaken.

ing, duplicating in every respect except temperature the experiments to be described below. In none was any decrease of ascorbic acid concentration detected in either true or separated serum.

It has already been shown and new evidence is presented that, when whole blood is allowed to stand at room temperature in contact with air, no loss of ascorbic acid can be demonstrated, even after several hours (1). When blood was kept at 37° C., slight losses occurred. Appreciable losses, however, were observed at this temperature when the blood was agitated continuously. It was found that if the blood was kept under an atmosphere of nitrogen these losses were entirely prevented. Observations on the stability of ascorbic acid added to defibrinated blood under these various conditions are shown in Table I.

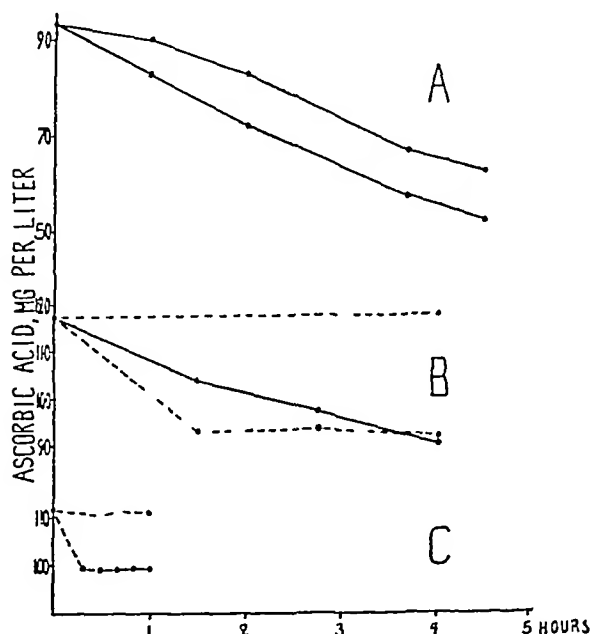


FIG. 1. CONDITIONS INFLUENCING THE CONCENTRATION OF ASCORBIC ACID IN "SEPARATED" AND "TRUE SERUM" AT 37° C.

Ascorbic acid added at zero time. Solid circles represent separated serum, open circles true serum, solid lines indicate that the blood was allowed to stand without motion, broken lines that it was continually agitated. A under air, B and C under nitrogen.

Figure 1 A depicts the results of one typical experiment out of 14 that agreed in all respects. The separated serum were kept exposed to air at 37° C. It was consistently found that both the

separated and the true serum lost ascorbic acid progressively but that the loss from the separated serum was always less than that from the true serum. To determine if these losses could be due to conversion of ascorbic acid to the reversibly oxidized form which cannot be detected by the method applied, simultaneous analyses were made employing H_2S according to the method of Emmerie and van Eekelen. From these comparisons it appeared that the losses in both separated and true sera were real ones. Experiments number 3, 4, and 5 of Table I show that ascorbic acid is protected in whole blood to some extent. The losses observed in whole blood during the first 3 hours can in no way account for the far greater decrease in ascorbic acid concentration taking place in true serum during the same period. These experiments therefore indicate that ascorbic acid has entered the cells. Neither by extending these experiments over periods as long as 9 hours nor by determining the ascorbic acid content in both sera at intervals as short as 15 minutes was any additional information obtained.

TABLE II

Effect of nitrogen on the stability of ascorbic acid in serum at 37° C.

Experiment number	Time of incubation	Separated serum	True serum
	minutes	mgm. per liter	mgm. per liter
1	0	108.5	79.0
	240	106.5	
2	0	117.0	75.5
	240	114.5	
3	0	100.5	84.0 70.0 68.0 66.0
	90		
	135		
	180		
	240	98.0	

The protection against loss of ascorbic acid by an atmosphere of nitrogen, shown before for whole blood was also observed in solutions of pure ascorbic acid in 1 per cent trichloroacetic acid, which deteriorate rapidly in air. From the data presented in Table II and in Figure 1 *B* and *C* it is evident that ascorbic acid in separated serum is also protected.

While there is no appreciable loss of ascorbic acid from separated serum the concentration of

ascorbic acid in true serum decreases even in an atmosphere of nitrogen.

The effect of an atmosphere of nitrogen on the experimental results is twofold (1) it stabilizes ascorbic acid in separated serum, (2) the decrease in ascorbic acid concentration in true serum which was progressive under air, is self terminative.

The rate of entrance of ascorbic acid into the blood cells irregular in the observations thus far presented, was considerably enhanced by continuously shaking the blood. This agitation was effected in the incubator by placing the flasks on a rack oscillated so gently by a windshield wiper motor that the cells were never mechanically damaged. In order to avoid temperature changes* which were found to cause fluctuations in the curves in the beginning of this investigation the nitrogen was led into the incubator and was washed by passing through a bottle of distilled water. From this wash bottle the gas was conducted through a manifold to a series of smaller wash bottles each of which was directly connected with a 25 cc. Erlenmeyer flask containing 2 cc. of blood or serum. By means of control screws the pressure of nitrogen reaching the surface of the fluid in each flask could be kept approximately equal. During the first 15 minutes the pressure was kept relatively high. It was then partially reduced for the remainder of the experiment.

In Figure 1 *B* and *C* is presented evidence of the influence of shaking on the rate of disappearance of ascorbic acid from true serum. Figure 1, *C* representative of 5 such experiments shows that the passage of ascorbic acid into the cells is self terminative, ceasing within 30 minutes.

DISCUSSION

At 37° C., in all the experiments presented, the concentration of ascorbic acid added to whole blood decreases in serum which is kept in contact with cells (true serum). These losses in true serum are not due to reversible oxidation but must be attributed to passage of ascorbic acid from the serum to the cells. This entrance of ascorbic acid

* These temperature changes possibly indicate the cause for unexplainable fluctuations observed in previous work (1 p. 757) where temperature equilibrium was not rigidly controlled.

into the blood cells occurs also in an atmosphere of nitrogen, in which deterioration of ascorbic acid is prevented in whole blood as well as in separated serum, at 37° C, even when the blood is continuously shaken

The fact that the transfer of ascorbic acid, established at 37° C, is entirely prevented at 7° C, whether the blood is exposed to air or nitrogen, suggests its association with some metabolic activity or chemical reaction rather than simple diffusion. In this respect it is analogous to the transfers of phosphate which were investigated by Halpern (8)

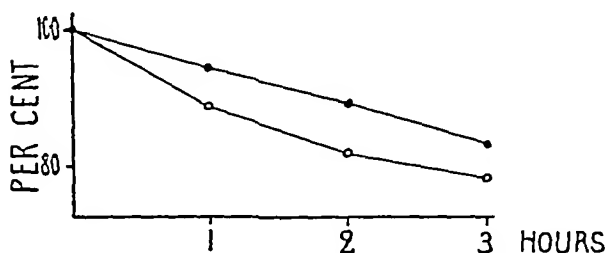


FIG 2 CONCENTRATION OF ASCORBIC ACID IN "SEPARATED" AND "TRUE SERA"

The curves represent the average of 14 experiments at 37° C. under air, expressed as per cent of the amount present immediately after addition of ascorbic acid at zero time. ●—● represents separated serum, ○—○ true serum.

Whether the rate of transfer and the amounts exchanged are different under air and nitrogen is as yet undecided. In Figure 2 the trends of the curves representing separated and true sera kept under air show that the rate of deterioration in the former is almost linear, in true serum, however, the ascorbic acid concentrations decline faster than in separated serum during the first hour of the experiment. Subsequently, the two curves run more or less parallel. From this observation it seems that, under air, in addition to the amounts which enter the cells, a portion of the ascorbic acid is destroyed. The destructive process appears to be continuous, while the transfer is self-terminative just as it is under anaerobic conditions. The deterioration and the transfer of ascorbic acid are both accelerated by continuous shaking, in one typical experiment after only 45 minutes the concentration in true serum decreased from 106.0 mgm per liter at zero time to 83.0 mgm per liter, and that in separated serum to 90.0 mgm per liter,

the corresponding data for sera kept under nitrogen were 94.0 mgm per liter and 106.5 mgm per liter

The experiments under air leave no doubt that ascorbic acid enters the blood cells. In the 5 experiments under nitrogen, of which Figure 1, C, is typical, and in which the concentrations of ascorbic acid in serum were raised to between 100 and 120 mgm per liter, the decrease in true serum varied from 11.3 to 19.2 per cent of the initial concentration. In 3 of these experiments, for which blood of the same person was used, the decrease varied from 11.3 to 12.1 per cent. Quantitative deductions as to absolute amounts require more data than are at present available. In two instances where different amounts of ascorbic acid were added to samples of the same blood, the absolute amounts taken up by the cells diminished as serum concentrations decreased. Estimations of the concentrations of ascorbic acid per unit of water in serum and cells indicated that at high concentrations the distribution ratios (calculated as $\frac{\text{Ascorbic acid of serum}}{\text{Ascorbic acid of cells}}$) were greater than unity, approaching unity at lower concentrations—i.e., about 35 mgm per liter of water

SUMMARY AND CONCLUSIONS

- 1 After addition of ascorbic acid to defibrinated human blood, its concentrations were followed in serum separated at once and in serum left in contact with cells
- 2 At 37° C, ascorbic acid enters the blood cells
- 3 This transfer is self-terminative and occurs under an atmosphere of air as well as of nitrogen, the latter preventing deterioration of ascorbic acid in serum and in whole blood
- 4 The passage of ascorbic acid into the cells appears to be associated with some metabolic activity since it was never observed at lower temperatures
- 5 The rate of transfer of ascorbic acid is enhanced by avoiding sedimentation of the blood

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RENAL BLOOD FLOW IN WOMEN WITH HYPERTENSION AND RENAL IMPAIRMENT

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Spontaneous hypertension in man has been divided into two main categories—the renal and non renal. Renal hypertension has been considered to arise secondarily to a kidney lesion or deficiency while the non renal (primary or essential) hypertension has been thought to exist without underlying kidney pathology (Alibutt Huchard, Jane-way cited by Fishberg (1)).

Goldblatt (2) and others using his method, have produced an experimental hypertension in dogs and monkeys by partial occlusion of the main renal arteries. Hypertension in conjunction with mechanical obstruction to the main renal arterial blood flow has been described as a clinical entity (3 and others) but nature's imitation of the experimental method is probably unusual. When interference with the renal blood flow exists it is more usually to be found in the arterioles glomerular capillaries and peritubular blood vessels (as in urinary back pressure).

Many studies have been made of the renal function in patients with essential hypertension and with the development of each new test more sensitive than its predecessors the field has been re-investigated. The characteristic finding in the early stages of the hypertension at least has been "normal" kidney function as measured by the test under consideration. However the tests have not been delicate enough to give the crucial answer, as will be discussed below. Recently Smith Goldring and Chasis (4) have defined the conditions under which the diodrast clearance is a measure of the renal plasma flow at low plasma levels of diodrast, the renal extraction ratio is very close to 1 in normal man. While a low diodrast clearance might be suspect on the ground that an impairment in renal efficiency could change the extraction ratio the clearance will always reflect the lowest possible volume of plasma which could have perfused the kidney during the clearance period. A normal diodrast clearance, therefore must mean a normal

plasma (and whole blood) flow through the kidneys during the test.

In the present report, we shall describe our measurements of renal blood flow in patients with considerable renal impairment with and without hypertension, and in patients with "essential" hypertension, some of whom had toxemia of pregnancy as the initial phase of the permanent hypertension. The questions chiefly to be considered are whether hypertension is always accompanied by a decrease in the total renal blood flow, and whether diminished renal blood flow is always accompanied by hypertension. Some inferences will be drawn as to the site and nature of vascular changes in the kidney itself.

MATERIAL AND METHODS

Ten patients with renal impairment were drawn from the postpartum toxemia clinic. By a "patient with renal impairment" we mean one in whom the urea clearance is consistently below 60 per cent of average normal, regardless of the cause.

The essential hypertension cases are divided into 2 groups. The post toxemic group of 16 patients was taken from the postpartum toxemia clinic. The other group of 11 subjects had no background of toxemia. These latter were drawn from the wards of the Jersey City Medical Center. By "essential hypertension" we mean the occurrence of increased blood pressure (usually marked) with minimal or no proteinuria, edema or hematuria, and with the urea clearance consistently above 70 per cent of average normal. Patients without a history of toxemia of pregnancy denied such history by name and by symptoms. In 2 cases it was possible to corroborate the patients' denials by contact with physicians, 2 patients had never been pregnant, 1 was a man, and all others were at least 12 years postpartum.

The effective renal blood flow was calculated by dividing the plasma fraction into the plasma clearance of diodrast (4). This measurement will hereinafter be called simply the renal blood flow. All figures are corrected to the ideal surface area of 1.73 sq. m. Details of the procedure, slightly modified from the description given by Smith, Goldring and Chasis (4) have been outlined in previous publications (5, 6). Briefly a 10 per cent solution of dextrose, containing 1 ml. of 35 per cent diodrast per 100 ml., is given intravenously at

the rate of 10 ml per minute for 10 minutes. The rate of infusion is then cut down to 4 ml per minute, at which rate it is continued throughout the test. About 30 to 40 minutes after the beginning of the infusion the bladder is washed out twice with physiological saline, using a special multi opening catheter. Urine collections are then made at intervals of about 30 minutes, the bladder being washed out twice each time. Usually 4 urine collections are made. At 45 minute intervals during the test, blood samples (oxalated) are collected for analysis, 4 samples are taken.

Diodrast in plasma and urine was analyzed as iodine, using a modification of Kendall's method (5, 6). All determinations were done in duplicate.

Simultaneous determinations were made of the plasma clearance of urea, using Van Slyke's manometric methods (7). The hematocrits were determined in vaccine tubes designed by MacKay for protein estimation (7).

RESULTS

Since our first publication (5), we have done diodrast clearances on 13 additional normal women. The renal blood flow in the series of 30 women ranges from 694 to 1233 ml, and averages 850 ml per minute per 1.73 sq m body surface. The standard deviation is 113 ml, and the standard error of the mean is 20.6 ml. Assuming that 60 per cent of the filtered urea is excreted in the final urine (in maximal urea clearances), a rough estimation of the glomerular filtration may be made from the urea clearance. Dividing this estimated filtration by the diodrast clearance (plasma flow) will give an approximation to the filtration fraction (*i.e.* the proportion of the perfusing plasma which is filtered through the glomerular capillaries). In our normal series, the filtration fraction thus estimated varies from 15.2 to 24.5 per cent, and averages 20.4 per cent. The standard deviation is 3.0 per cent, and the standard error of the mean is 0.65, 21 cases are analyzed (Two atypical cases with very high urea clearances are not included). All data for normal subjects are given in Table I.

Our filtration fraction for normal women is somewhat higher than Smith's (8), which averaged 17.7 per cent for men. In this connection, it is interesting to note that our average plasma urea clearance for women is identical with Smith's average for men, at 70 ml per minute. At the same time the renal blood flow in our women is considerably below the level which Smith reports for men (846 ml and 1275 ml, respectively). This

TABLE I

Plasma clearances of diodrast and urea, urea extraction ratios, estimated filtration fractions, and renal blood flows in 30 normal women

Filtration fraction estimated from maximal urea clearance, which is assumed to be 0.60 of the glomerular filtration rate

Plasma clearance of		Urea extraction ratio	Filtration fraction	Renal blood flow	
Diodrast	Urea				
<i>ml per minute</i>	<i>ml per minute</i>	<i>per cent</i>	<i>per cent</i>	<i>ml per minute</i>	
PREGNANT, NEAR TERM					
520	(91 0)*	(11 6)*	(29 2)*	787	
848	(138 0)*	(11 2)*	(28 9)*	1233	
560	64 2	8 6	19 1	793	
513	47 5	6 9	15 5	694	
607				886	
672				995	
593				833	
568	82 8	10 2	24 2	812	
536	67 8	8 8	21 1	770	
521	50 5	6 9	16 2	733	
619				848	
674	96 4	9 9	23 8	971	
538	70 6	8 6	21 9	821	
613	74 0	8 0	20 1	925	
479	70 4	9 5	24 5	742	
NON PREGNANT					
461	49 4	6 6	17 7	752	
531				792	
542	48 1	5 7	15 2	851	
485	71 0	9 3	24 4	769	
676	97 4	9 4	24 0	1029	
601	61 0	5 8	17 0	1049	
554	65 6	7 2	19 7	918	
501	61 3	7 7	17 3	807	
524	71 3	8 5	22 7	846	
461				738	
721	98 0	10 0	22 7	983	
493	60 9	8 8	20 6	693	
459	45 9	6 3	16 7	731	
525	75 4	9 3	23 9	811	
607				886	
Mean	567	70 0	8 3	20 4	850
σ	82 5	15 1	1 6	3 0	113
S E \bar{x}	15 1	3 3	0 35	0 65	20 6

* Not averaged because the blood urea was considered to be erroneously low, plasma urea N/N P N ratios less than 0.28

necessarily means that in our women the urea extraction ratio is higher than in Smith's men. This is effected by two means: the filtration fraction is higher in women, and the hematocrit is lower. That is, more of the woman's blood is plasma, and a larger proportion of plasma is filtered at the glomerulus.

Since our findings in cases of hypertension following toxemia of pregnancy usually showed certain qualitative as well as quantitative differences from the results reported by Smith (8) for hypertension in men we have measured the renal blood

TABLE II

Hypertension renal impairment and renal blood flow

Blood pressure readings made during tests. Filtration fraction approximated from maximal urea clearance which is assumed to be 0.60 of glomerular filtration. Renal ischemia in percentage represents the deficit in renal blood flow as compared with average normal of 850 ml. per minute

Age	Post partum	Blood pressure	Plasma clearance of		Urea excretion ratio	Filtration fraction	Renal blood flow	Renal ischemia
			Diodrast	Urea				
years	years	mm. Hg	ml per minute	ml per minute	per cent	per cent	ml per minute	per cent

A. HYPERTENSIVES WITHOUT HISTORY OF TOXEMIA OF PREGNANCY

45	N*	216/100	273	54.1	12.9	33.0	420	51
50	N*	224/108	401	79.6	12.6	33.1	629	26
22	♂	150/110	90†	19.0†	12.4	32.8	153†	74
29	6.2	240/160	218	59.4	16.2	45.4	366	57
49	25.0	186/110	188	34.0†	10.8	31.4	315	63
50	12.0	210/110	330	67.6	7.8	21.5	868	0
50	22.0	206/110	357	72.7	8.5	21.7	860	0
49	20.0	160/110	328	54.4	11.7	27.6	467	45
47	21.0	204/110	543	77.0	9.0	23.6	859	0
48	17.0	234/134	322	49.7	9.4	25.7	531	36
54	19.0	230/145	401	60.0	8.8	25.0	685	20

B. HYPERTENSIVES WITH HISTORY OF TOXEMIA OF PREGNANCY

41	0.1	170/106	358	68.0	13.6	32.0	503	41
30	1.3	192/120	434	48.1	6.9	18.5	700	18
34	3.2	152/90	403	52.0	8.7	21.4	597	30
27	3.4	180/130	338	58.4	7.1	18.1	828	2
48	4.7	168/90	430	43.2	5.6	16.8	765	10
37	2.2	170/120	437	70.2	10.0	26.9	705	17
48	3.6	150/90	517	62.2	7.6	20.0	815	4
38	5.5	210/140	376	38.3	6.7	17.0	567	33
40	2.0	240/126	450	57.9	9.0	21.5	648	34
29	1.8	220/120	377	40.5	6.8	17.9	599	30
48	5.0	200/110	382	38.6	6.6	16.8	585	31
40	4.0	190/150	349	47.5	9.3	22.7	512	40
30	0.6	180/120	454	53.2	7.7	19.5	690	10
24	0.1	170/100	531	63.0	8.2	19.8	766	10
35	1.3	140/90	392	54.9	9.0	23.3	608	28
43	8.0	214/140	160	30.4	8.6	(31.4)‡	350	59

C. PATIENTS WITH RENAL IMPAIRMENT

26	0.3	90/60	300	34.0	8.3	18.8	413	51
20	2.4	100/60	330	38.5	7.8	19.4	495	41
28	1.2	150/94	184	17.7	6.4	15.9	279	67
29	3.3	70/50	275	34.7	8.0	21.0	433	49
27	ante-partum	100/60	156	21.8	10.8	25.3	201	76
28	ante-partum	155/100	70	9.5	9.7	22.6	98	88
34	2.8	120/80	219	25.1	7.4	19.1	338	60
32	3.5	120/90	435	36.0	5.7	13.9	636	25
23	0.1	200/130	290	29.4	9.8	16.8	432	49
43	0.1	156/90	342	32.8	7.5	17.5	439	48

* Nulligravida.

† Left kidney only (right kidney had undergone sympathetic denervation)

‡ Standard urea clearance.

§ High because of very high hematocrit (55 per cent)

flow in 11 hypertensive patients who had no toxemic pregnancy. In these few cases with 3 exceptions, our data confirm Smith's findings. As shown in Section A of Table II a diminished blood flow is present in all but 3 cases. Moreover the filtration fraction is characteristically elevated (though not in the subjects with normal renal blood flow). Smith has explained renal ischemia together with a high filtration fraction on the basis of efferent glomerular arteriolar constriction. As he has pointed out, this maintenance of a high filtration fraction accounts for the frequent finding of "normal" renal function as shown by the usual tests even with a great deficit in the volume of blood perfusing the kidney.

The findings in the majority of our post toxemic hypertensive patients do not conform with the above conditions (Section B of Table II). In most cases there is a diminution in the renal blood flow as compared with the average normal. Yet 8 of the 16 values are in the normal range as given by the normal mean minus twice its standard deviation. Significantly the filtration fraction is not increased (2 exceptions). In other words, the urea clearance parallels closely the renal blood flow as calculated from the diodrast clearance. In a few of these cases, the urea clearance (Table II) is subnormal, however in nearly all of these patients the urea clearance is usually 70 per cent (49 ml per minute) or more of normal.

Two of the 4 hypertensive patients showing absolutely no diminution in total renal blood flow (3 in Section A and 1 in Section B of Table II) had at the time of the test blood pressure readings far lower than the usual levels observed. The first patient's pressure often exceeded 300 mm Hg in the systolic and 150 mm Hg in the diastolic readings. The fourth patient's pressure usually ran about 270/160. During the tests under basal conditions the blood pressures in these women were 210/110 and 180/130 respectively. Perhaps if the renal blood flows were measured at another time, with the higher blood pressures, some deficit might be found (if increased tension did not compensate for the narrowed arterioles and maintain normal renal blood flow). In both of these cases the filtration fraction was normal at the time of observation. The blood pressures of the other patients were at the usual levels, even after bed rest of several days.

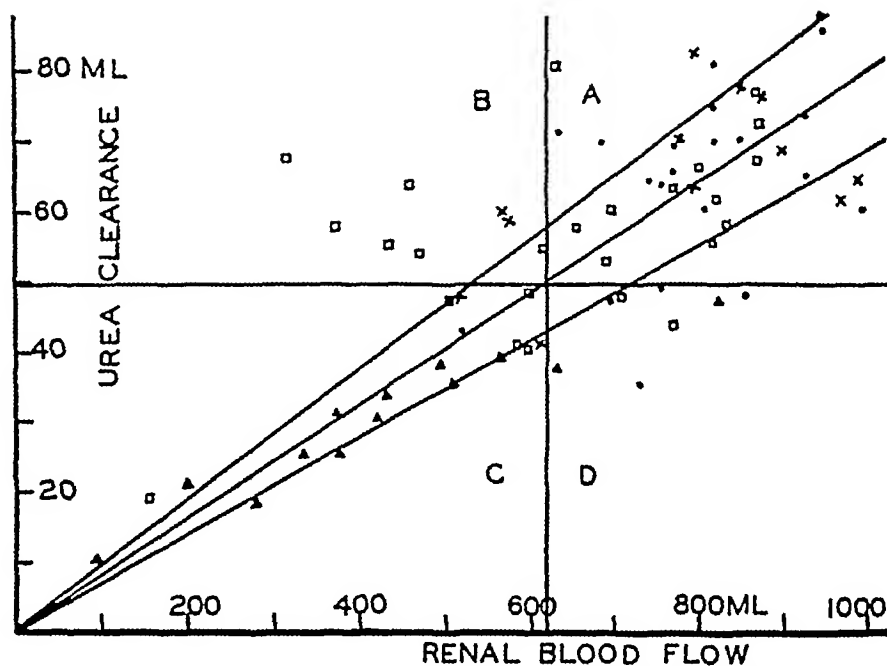


FIG 1 THE RELATIONSHIP BETWEEN THE UREA CLEARANCE AND THE RENAL BLOOD FLOW

The graph is divided into 4 squares by lines representing the lower normal limits of urea clearance (49 ml per minute) and of the renal blood flow (625 ml per minute) See text for explanation.

- Normal women
- × Preeclamptic and eclamptic women (previously published (6))
- Hypertensive women
- ▲ Women with renal impairment (3 cases published (6))

Data for the previously published cases are not presented in Table II Seventeen of the normal subjects formed the basis for an earlier publication (5)

One may now ask whether diminished renal blood flow, when present, must cause hypertension (We are not equating diminished renal blood flow with renal ischemia See Discussion) As an approach to this question we have measured the diodrast clearance in a selected group of 10 patients with definite renal impairment, the majority of whom maintain normal blood pressures As stated above, the renal impairment in different cases is attributable to diverse causes For the moment it will be assumed that the diodrast clearance is complete in these patients, and is therefore a measure of the renal plasma flow The observations on these patients are shown in Section C of Table II In every case there is a marked diminution in the diodrast clearance and presumably, therefore, in the renal blood flow The filtration fraction was always found to be in the normal range. In the cases showing increased blood

pressure, the filtration fraction was normal or even a little low The urea clearance, therefore, closely parallels the diodrast clearance and renal blood flow over the whole range down to about 10 per cent of normal function

A comparison of the urea clearance with the renal blood flow (calculated from the diodrast clearance) is plotted in Figure 1 The 70 subjects represented comprise normals, as well as patients with toxemia of pregnancy (5, 6), post-toxemic hypertension, chronic nephritis, pyelonephritis, and post-toxemic renal damage As may be seen from the graph, the parallelism is good, and the urea clearance follows fairly closely the renal blood flow In quantitative terms, the coefficient of correlation (r) between renal blood flow and urea clearance is 0.79 for all of these subjects (A few subjects with very high urea clearances, up to 200 per cent, had correspondingly

high renal blood flows, these are not shown in the graph.)

Figure 1 has been subdivided into 4 squares by representing the lower limits of normal urea clearance and of renal blood flow. These lower normal limits have been set by subtracting twice the standard deviation from the mean. The positive lines, originating at zero describe the average ratio between the urea clearance and renal blood flow (middle line) and include points falling within plus or minus 15 per cent of the average ratio (outside lines). It will be seen that in most cases one may predict within 15 per cent, the renal blood flow from the urea clearance. The graph might be further subdivided into 10 fields with a different interpretation for a point falling in any one of these fields. For the sake of brevity we shall consider only the 4 major squares. *A* is the field of normal kidney function. *B* represents diminished renal blood flow with normal urea clearance (normal filtration), high filtration fraction and probable efferent glomerular arteriolar constriction. Smith's (8) male hypertensives fall in this field, as do all but 3 of our hypertensive women who had no history of toxemia of pregnancy. Two cases of toxemia of pregnancy, 1 an active eclamptic, also fall in this area. *C* includes cases of renal impairment. When the points fall above the upper positive line originating at zero constriction of the efferent glomerular arteriole is present and compensates in part for the diminution in renal blood flow. Essential hypertension patients with renal impairment would conform to the latter description. *D* describes cases in which the urea clearance is disproportionately low as compared with the renal blood flow. In our series, the patients falling in this category were varied. There were 4 normals presumably showing low variations in the urea clearance, which does fluctuate widely in normal subjects. There were 2 post toxemic hypertensives and 1 toxemia of pregnancy for which we offer a possible explanation in the Discussion below. The 2 cases of 'renal impairment' had pyelonephritis. Ascending infections of the urinary tract are thought to permit increased back diffusion of urea through the tubular epithelium when inflammation involves these structures.

DISCUSSION

We have previously shown that the renal blood flow, as measured by the diodrast clearance, is normal during the hypertension found in the acute phases of the toxemias of pregnancy. As shown by the cases described above and summarized in Table II many cases of post toxemic hypertension fall in the normal range of renal blood flow (as does an occasional case of hypertension with no history of toxemia). When the renal blood flow is diminished in these cases the decrease may perhaps be secondary to anatomical changes. In practically all of this group of patients the hypertension is known to have existed for several years. This is partially indicated by the "years postpartum" column in Table II. All patients had had hypertension for the period indicated there (Section B), and many were known to have had hypertension antedating the most recent pregnancy from which years postpartum is reckoned. In brief, the hypertension in some cases does not seem to depend upon a reduction in the total volume of blood perfusing the kidneys. (Whether diminution in blood flow is the mechanism by which Goldblatt clamps cause hypertension is an open question. Apparently, there are no published data on the renal blood flow in Goldblatt animals in which the arterial constriction is just luminal.)

Moreover diminished renal blood flow *per se* does not seem necessarily to result in hypertension, as witness the cases in Section C of Table II. There are complicating factors to be considered before drawing this conclusion. First, one may ask is the renal extraction ratio for diodrast unchanged from the normally complete extraction? That is does the diodrast clearance really measure the renal plasma flow in these cases of renal impairment? We can give no definite and direct answer to this question. Urea is excreted wholly by glomerular filtration while diodrast is secreted chiefly by the tubules (at least 80 per cent by tubular secretion). Yet taking all of our cases together over the very wide range of from 10 to 200 per cent of average normal renal function, the clearance of diodrast and of urea is closely parallel. This suggests a common controlling factor in the renal blood flow. In the dog the urea

clearance parallels the renal blood flow, as Van Slyke, Rhoads and Hiller (9) have demonstrated. Furthermore, these subjects showed renal impairment by all tests of renal function, and it might be surmised that destruction of renal substance had occurred, thus necessarily reducing the blood flow and diodrast clearance.

Assuming a complete clearance of diodrast, the renal blood flow, then, would be markedly diminished in these patients with renal impairment. But is there renal ischemia in the sense that intact renal parenchyma is inadequately supplied with blood? Or is the renal blood flow merely lessened in proportion as renal parenchyma is destroyed, with the remaining tissue receiving a normal amount of blood? We have no answer for these questions. In glomerulonephritis it is probable that the glomerular lesions would impede the flow of blood and thus partially shut off the supply to the tubules. In the cases of post-toxic renal damage, different glomerular lesions may cause the same thing to happen.

The glomerular lesion in toxemia of pregnancy has been described by Bell (10) as a thickening of the basement membrane in the glomerular capillaries. Baird and Dunn (11) have confirmed this, and recently Page and Cox (12) have found the same pathology persisting several years until death from various other causes. Possibly this thickening of the basement membrane may explain in part our findings in post-toxic hypertensives. These findings include (1) renal ischemia when present, (2) the parallelism of the urea clearance (and glomerular filtration) with the renal blood flow, and (3) the not uncommon tendency of some of these patients to maintain for years a constant low level of renal function.

Since about one-fourth of the cardiac output perfuses the kidneys, which constitute less than 0.5 per cent of the body weight, the resistance here to blood flow must be relatively slight. If the glomerular capillaries were even slightly narrowed by the thickened basement membrane, the resistance to perfusion would be increased, and the total volume of fluid flow lessened. The minimum effect of such luminal narrowing of the capillaries may be approximated from Poiseuille's law which gives the volume (Q) of fluid perfusing a capillary tube in unit time (T) as

$$Q = \frac{\pi p r^4}{8 l \eta} t,$$

where p is the pressure decrement in the length (l) of the tube, the radius of which is r , η is the coefficient of viscosity. The factors π , 8 and t are, of course, constant. Assuming for the moment that p and η are also constant, a diminution of 10 per cent in the capillary diameter would decrease the blood flow by 35 per cent. The effective viscosity (η) must be increased, which would reduce still more the blood flow. While Fåhræus and Lindqvist (13) have shown that the coefficient of viscosity for blood decreases when the diameter of the tube falls below 0.3 mm, their data are not applicable to blood flow in capillaries where the diameter of the red blood corpuscles so closely approaches the diameter of the vessel that smoothly moving concentric layers cannot be formed with the cells in the axial stream (14). The narrowing of the capillaries may, perhaps, be partially offset by a greater pressure decrement. When the compensation is not complete, a decrease in blood flow would result. We shall return to this factor presently.

Thus any appreciable narrowing of the capillaries may be presumed to diminish the volume of blood circulation. This would account for the renal ischemia, when ischemia is present. Since the ischemia does not seem to be caused by, or associated with, constriction of the efferent glomerular arteriole, intra-glomerular pressure is not markedly increased, the filtration fraction remains normal, and the glomerular filtration (and urea clearance) varies with the renal blood flow. Two of our 14 post-toxic hypertensives do show high filtration fractions, but the usual case apparently does not conform with Smith's findings in male hypertensives.

As for the third observation, which may be explained on the basis of the thickened basement membrane, most of the patients in Sections B and C of Table II, as well as many others not included in this study, have been followed for several years, in that time the renal functional levels have either not changed at all, or have shown very slow improvement. This has been found in patients with urea clearances as low as 20 per cent, and urea clearances have remained at that level for as long as 5 years. When the urea clearance falls

as 20 per cent in chronic glomerulonephritis or in nephrosclerosis the renal lesion progresses. In acute nephritis the lesion either regresses or goes on to a fatal outcome. Conceivably, during toxemia of pregnancy the glomerular lesion is produced. With termination of the pregnancy, and of the toxemia, further damage to the glomerular capillaries does not occur and renal function becomes stabilized. In a few cases a very slow improvement in function may occur in some malignant nephrosclerosis supervenes (15). Some of our cases have been reviewed elsewhere (16) in another connection and without consideration of the specific lesion which may underlie the deficit in kidney function.

It would appear from the above considerations that post toxicemic hypertension is often on a different basis from the hypertension found in men (Smith) and in some women who have not had toxemia of pregnancy (Section A, Table II) though the dividing line is not a hard and fast one. Therapeutic measures designed to relax the constriction in the efferent glomerular arteriole may be beneficial in the latter group. Perhaps such treatment would be ineffective in post-toxicemic hypertensives.

Arterial hypertension has often been regarded as a compensatory mechanism which maintains a relatively normal glomerular filtration either in the face of a deficit in renal flow, or by increasing the volume of blood perfusing the kidney. In the first case, if the increased systemic blood pressure is reflected in an increased intra glomerular pressure, the filtration fraction should be increased. This factor together with efferent arteriolar constriction has been mentioned by Smith as accounting for the high filtration fractions seen in male hypertensives. In the second case if hypertension increases the renal blood flow by driving the blood through the kidney at an increased rate, and by providing a high head of pressure permitting of an increased decrement (p in Poiseuille's equation), one may conceive that the filtration fraction might not be increased even with a high intra glomerular pressure. The plasma in a given volume of blood may simply be too short a time in contact with the filtering surface if the resistance to flow is not distal to the glomerulus. Yet because of the increased volume of blood passing through the glomerular capil-

laries, the total filtration is increased by hypertension. While a quantitative separation of these two effects of hypertension is impossible, it may be that each of our 2 groups of hypertensives shows predominantly one and the other of these compensatory mechanisms. The patients of Section A of Table II and the male hypertensives described by Smith (8) show high filtration fractions. The constriction of the efferent glomerular arteriole imposes a resistance to blood flow distal to the glomerulus and thus slows the rate of flow through the glomerular capillaries. The arterial hypertension reflected in increased intra-glomerular pressure raises the filtration. In the post toxicemic hypertensives the resistance to perfusion may be in the glomerular capillaries whose lumina are narrowed by the thickened basement membrane. Hence, it is through the capillaries that the blood must flow most rapidly. Hypertension may be the propulsive force which keeps the total volume normal or nearly so by speeding the flow through the point of resistance. The filtration which occurs through the capillary walls may be lessened because of the thickened filter. Because of this and because of the shorter time of contact of a unit volume of plasma with the filter the filtration fraction tends to fall, but the tendency is offset by the higher pressure. Hypertension thus maintains the glomerular filtration at normal levels by opposing the resistance to perfusion and thus increasing the renal blood flow.

Finally, it is worth mentioning that, except in certain cases of essential hypertension the urea clearance is as good a test of renal function as is the diodrast clearance. The more one uses the urea clearance the more one is impressed with its clinical value. As has long been known, the urea clearance usually is proportional to the glomerular filtration and therefore gives an estimate of glomerular function. As our data demonstrate, the urea clearance also will give an estimate of the renal blood flow. Landis, Elsom, Bott and Shuels (17) have shown the same parallelism between the plasma clearances of urea and hippuran the latter of which approaches the complete clearance of diodrast.

SUMMARY AND CONCLUSIONS

Using the diodrast clearance indirect measurements of the effective renal blood flow have been

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THE INFLUENCE OF VITAMIN A UPON UREA CLEARANCE IN THE HUMAN SUBJECT¹

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The object of this study was to ascertain the effect upon the urea clearance of the addition of large amounts of vitamin A to the ordinary mixed diet of normal subjects

METHODS

The subjects presented no evidence or history of serious renal disease. Nine of the subjects were male medical students. Subject 4 was a 48-year-old female patient, showing severe signs of vitamin A deficiency. Three were staff members of the physiology department. The clearances were conducted about 15 hours after the last meal. Approximately a liter of water was drunk about an hour before the clearance periods. During the clearance periods from 500 to 1000 cc. of water were taken. Urine collection was not begun until 200 cc. of urine had been passed. This eliminated the possibility of washing out concentrated urine from the dead space of the urinary system. Periods in which the rate of urine flow was rising were avoided or rejected. In the first 7 subjects in Table I the bladder was catheterized and washed with sterile saline before and at the end of each collection period. In the remaining subjects, the bladder was not regularly catheterized because the maximum retention after voluntary micturition was never found to be greater than 5 cc. and with a urine volume between 150 cc. and 400 cc. the error was negligible. The length of the collection periods varied from 30 to 45 minutes. Venous blood was drawn at the mid point of the collection period. Urea was determined by the urease method as outlined by Peters and Van Slyke (1). The clearances are expressed in cubic centimeters of plasma cleared of urea per minute and are maximum clearances.

The normal clearances for Subjects 1 2 3 and 5 were made over a period of 3 weeks. Subject 4 was only available for 2 weeks. After the controls were obtained, vitamin A was administered daily. Subject 1 ingested capsules of carotene in oil equivalent to 50,000 international units of vitamin A per day. Subjects 2 and 3 ingested the non-saponifiable fraction (N.S.F.) of halibut liver oil in Wesson oil equivalent to 45,000 units of vitamin A for 25 days, after which it was raised to 96,000 units of vitamin A. Subject 5 received the N.S.F. of halibut liver oil equivalent to 175,000 units of vitamin A. Subject 4 received carotene, equivalent to 50,000

units and the N.S.F. equivalent to 100,000 units of vitamin A per day. Subjects 7 8 and 9 received 50,000 units of vitamin A in the form of halibut liver oil. Subjects 6 10 11 12 and 13 received 60,000 units of vitamin A in the form of halibut liver oil. No attempt was made to regulate the other portion of the dietary.

Serum vitamin A was determined in the first 9 subjects, using the procedure of van Eekelen (2) and the Lovibond tintometer. In the remaining subjects serum vitamin A was estimated by the procedure of Evelyn (3) and the photoelectric colorimeter. The serum samples were taken at the time of the clearance determination. The vision of 4 subjects in dim light, as tested by the bio-photometer was followed throughout the experiment by the courtesy of Dr. Horace Getz. The visual measurements are expressed in millifoot candles. The smaller the number the better the dim vision. Oxygen consumption was determined by the Sanborn apparatus either during an actual clearance run or under similar conditions with the subject recumbent.

RESULTS

The normal clearance values are shown in Table I. The clearances are designated normal in the sense that there was no evidence of renal pathology and no particular attention had been given to the dietary vitamin A. However, Subjects 4 5 7 8 9 and 10 have been found by Dr. Getz of the Department of Bacteriology to have subnormal dim vision as determined with the bio-photometer. The clearances listed under vitamin A supplement are the highest values obtained on any one half day and with the exception of Subjects 4 5 6 and 7 two sets of values are included. Figure 1 shows all clearances as determined on 4 subjects before the vitamin A supplement was begun during its administration and after its withdrawal. The serum for the vitamin A values in Table I was drawn at the time of the clearance determinations in Tables I and II.

The vitamin A must be administered for 2 or 3 weeks before any significant increase in urea clearance appears. About 8 weeks are required for the maximum response. This is illustrated by Subjects 1 and 2. After administration of the vitamin A supplement for 13 days the increases

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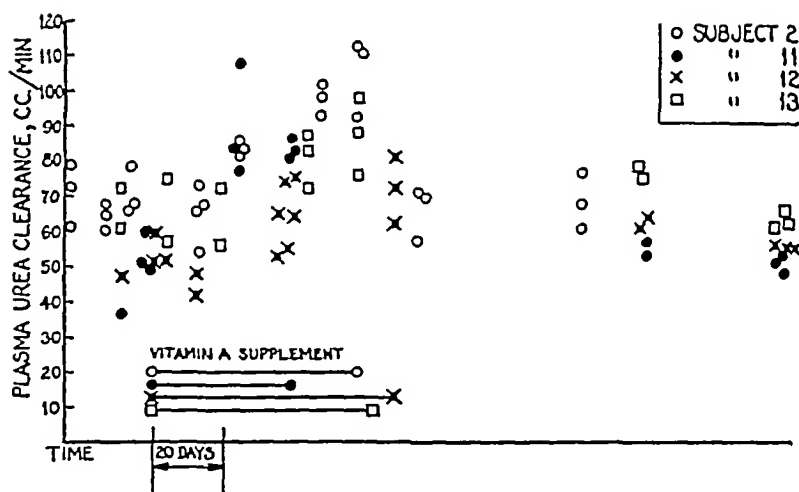


FIG 1 UREA CLEARANCE BEFORE, DURING AND AFTER THE VITAMIN A SUPPLEMENT

A comparison of the effects of the vitamin A supplement upon serum vitamin A, dim vision and urea clearance is seen in Table II

COMMENT

Although vitamin A does increase urea clearance in nearly all cases, the individual variability is striking and we have no explanation for it. Such variability was not found in the case of the dog (4) or rat (5). The limited data agree with the idea that the variability is associated with the constitutional type. The individual with the greater amount of subcutaneous fat, who gains or loses weight easily, is the type who is most likely to show an increase in urea clearance with vitamin A administration. It is interesting in this connection to note that, in the experimental animal, loss of subcutaneous fat is one of the early signs of avitaminosis. In 2 subjects the increased clearance was not accompanied by significant changes in arterial blood pressure or oxygen consumption. Therefore, changes in the general circulation are probably not responsible for the elevation of clearance.

As seen in Table II, improvement in dim vision does not necessarily accompany increased serum vitamin A or the elevation of urea clearance. Undoubtedly, many factors other than the supply of vitamin A enter into the rate at which visual purple is regenerated.

SUMMARY

Vitamin A in the form of a concentrate of halibut liver oil or the entire oil was administered in daily doses varying from 50,000 to 75,000 international units to 10 male and 3 female adults. In 2 subjects there was no appreciable change in urea clearance, in 4 subjects the clearance increased 11 to 15 per cent, and in 7 subjects the clearance elevation ranged from 24 to 91 per cent. The subjects in this latter group were the type who easily gain in body weight. The increase in clearance in 2 subjects was not accompanied by significant changes in blood pressure or oxygen consumption.

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BLOOD CHANGES INDUCED BY VENESECTION IN WOMEN WITH TOXEMIA OF LATE PREGNANCY

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The beneficial effect of blood letting in the toxemias of late pregnancy is well recognized, but little or no accurate information is available concerning the method of its action. During the course of investigations on the water concentration and the acid base balance in a series of patients with various clinical types of toxemia (1), certain observations were made at different times in the same individual in the hope of determining the physico-chemical effects of venesection. The methods employed were those previously described (2, 3), except that the pH was determined colorimetrically and corrected to the electrometric value by subtracting a determined factor, 0.34. In each case the pH of the two samples (the first and last portions of the venesection) was compared with the same standard and then one was compared with the other. The clinical data on the patients mentioned are available in Tables I, II, and III of the communication mentioned above (1).

Venesection was introduced into the therapy of the toxemias on an empirical basis in the belief that it would remove from the body some of the hypothetical 'toxin' supposedly responsible for the condition. More recently there has been a tendency to view its action as largely physical in nature. It is known that toxemic women with well defined clinical edema usually respond to therapy which invokes the mobilization of tissue water. The removal of large volumes (300 to 600 cc.) of blood increases the water content of the plasma and decreases the cell volume of the whole blood by drawing tissue water into the blood stream. This sequence of events is evidently based upon two physiologic facts: (1) the volume of circulating blood tends to remain constant, and (2) deficiencies in its volume are made up promptly by the withdrawal of tissue fluid when other external sources are unavailable. The fluid which is thus brought into the blood stream can then be eliminated by the kidneys, with the estab-

lishment of a mild diuresis. In all probability it is the extracellular tissue fluid which is first mobilized for this purpose.

Assuming the correctness of these observations, it would then appear that study of the first and last portions of a slowly performed venesection might give some indication of the composition of the tissue fluids.

One normal male control (a professional blood donor) and six toxemic patients (one hypertensive, three non-convulsive toxemics, and two eclamptics) were studied in this fashion, the amount of blood taken varying from 275 to 600 cc. and the time of withdrawal from 10 to 15 minutes. In certain instances a third specimen

TABLE I
Data on the details of the venesections

Case number	Hospital number	Amount of blood drawn	Time of venesection, minutes	Remarks
94a b		600	15	Professional male donor
89a b	D 5247	400	15	Toxemia without convulsions
90a b	H 13800	450	10	Toxemia without convulsions
91a b	J 1409	300	15	Chronic cardiovascular renal disease
92a b	J 5215	275	15	Postpartum eclampsia; the blood was drawn 4 or 5 hours after the last seizure
93a b c	J 5370	400 50	15	Eclampsia; blood drawing was made 1½ hours after the last seizure. The third specimen (c) was drawn 15 minutes after the venesection was ended
95a b c	J 6209	450 50	10	Toxemia without convulsions. The third specimen (c) was drawn 3 hours later after had been

TABLE II

Water distribution in the first (a) and the last (b), (c), portions of a venesection

Case number	Cell volume	Specific gravity			Water			Hemoglobin		
		Plasma	Whole blood	Cells	Per kgm. plasma	Per kgm. whole blood	Per kgm. cells	Per 100 cc. whole blood	Per kgm. cells	Per kgm. water
	per cent				grams	grams	grams	m. eq.	m. eq.	m. eq.
94a	48.0	1.0181	1.0477	1.031	912	792	665	10.12	19.50	29.35
b	46.0	1.0169	1.0425	1.073	916	797	663	9.63	19.34	29.50
82a	42.0	1.0192	1.0446	1.050	914	801	655	8.52	19.42	29.70
b	33.0	1.0160	1.0416	1.079	926	803	645	8.47	20.65	32.05
90a	27.5	1.0184	1.0366	1.056	910	855	713	4.13	14.22	20.00
b	24.0	1.0169	1.0356	1.083	916	858	695	4.03	15.46	22.25
91a	42.7	1.0125	1.0427	1.050	918	793	648	9.50	20.60	31.75
b	41.0	1.0140	1.0413	1.050	921	804	646	10.13	22.90	35.40
92a	31.5	1.0170	1.0377	1.055	916	835	668	6.38	18.65	28.00
b	30.5	1.0163	1.0365	1.052	918	833	668	6.25	18.92	28.41
93a	41.0	1.0160	1.0459	1.090	918	801	645	9.83	19.85	30.50
b	40.2	1.0157	1.0440	1.085	919	808	653	9.06	20.75	31.80
95a	48.0	1.0121	1.0459	1.088	903	783	641	10.12	20.21	31.55
b	43.0	1.0105	1.0433	1.089	912	791	639	9.51	20.30	31.75
c	42.5	1.0095	1.0395	1.079	914	792	635	8.75	19.10	30.05

was removed 15 minutes after the second. The data on the details of the venesection and the time of collection of the samples are recorded in Table I.

The water distribution in the various specimens is presented in Table II.

In the normal male subject it is apparent that after the venesection the cell volume and the various specific gravities are reduced, the water content of the plasma and whole blood is increased, while that of the red cells is practically unchanged, and the hemoglobin of the whole blood is appreciably reduced. It should be noted that the hemoglobin per kgm. of cells and of water is not altered.

In general, the results in the toxic women are of the same order and magnitude except that the hemoglobin concentrations per kgm. of cells and per kgm. of water are appreciably increased.

The acid-base factors are presented in Table III.

After venesection in the normal male, the chlorides, phosphates, and proteinates are decreased somewhat, while the bicarbonate rises fractionally, and the total acid is reduced slightly. On the basic side, all factors are lowered and the total base is reduced significantly. The pH and CO₂ capacity are practically unchanged.

Among the toxic patients, the changes are gen-

TABLE III

Acid-base equilibrium of the plasma before and after venesection

(Concentrations are reported in terms of milli equivalents, mEq., per kilogram of water)

Case number	(Cl) ⁻	(HCO ₃) ⁻	(Protein) ⁻	(HPO ₄) ⁻ + (H ₂ PO ₄) ⁻	Total acids [†]	(Na) ⁺	(Ca) ⁺⁺	(K) ⁺	(Mg) ⁺⁺	Total base	pH	CO ₂ capacity
	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.		Volume per cent
94a	103.2	30.7	21.9	1.74	158.3	153.1	5.48	5.44	2.19	166.2	7.45	68.0
b	102.9	31.4	20.6	1.70	157.4	145.5	4.97	5.37	2.18	158.0	7.48	68.9
89a	111.1	23.3	14.2	2.56	152.0	147.6	4.60	5.34		159.7*	7.16	53.0
b	109.9	20.9	13.4	2.44	152.0	144.9	4.45	5.52		157.1*	7.25	50.1
90a	114.0	18.5	16.6	2.27	152.2	151.0	5.06	5.45		163.7*	7.28	46.5
b	113.3	17.0	17.9	2.21	151.2	149.1	5.17	5.67		162.1*	7.41	45.8
91a	109.6	22.8	14.2	1.97	149.4	155.1	4.50	7.65	2.34	169.6	7.44	55.9
c	108.8	23.8	14.9	1.86	150.2	148.6	4.27	6.27	2.28	161.4	7.62	56.5
92a	105.8	27.0	19.2	2.60	155.4	140.0	4.65	8.74	2.41	155.8	7.44	62.0
b	104.0	25.8	19.0	2.63	152.2	141.9	4.65	9.12	2.18	157.9	7.50	59.0
93a	106.9	23.6	17.1	2.22	149.8	153.8	4.56	5.64	2.32	166.3	7.38	53.8
b	108.1	23.9	17.1		152.1‡	153.6	4.80		2.18	166.2‡	7.43	55.5
c	105.3	23.6	17.7	2.80	150.2	146.1	4.96	4.49	2.28	157.8	7.41	53.7
95a	102.2	26.6	18.1	3.89	151.6	146.9	5.16	5.50	2.57	160.1	7.44	59.6
b	103.0	26.5	17.7	3.48	151.5	141.6	5.16	5.50	2.31	154.6	7.48	59.2
c	103.9	27.0	16.7		151.9§	145.0	5.16	5.50		157.9*	7.44	61.5

* Assumed the value of 2.2 for magnesium

† Assumed the value of 5.64 for potassium

‡ Assumed the value of 2.2 for phosphates

§ Assumed the value of 3.48 for phosphates

|| Assumed the same water data as in 93c

§ Assumed the value of 0.8 for sulfates

erally in the same direction and of approximately the same extent except that the hydrogen ion concentration is invariably higher in the second specimen, and the variations are at times rather marked (0.13 in Case 90 and 0.18 in Case 91). This may be taken to indicate that the pH of the mobilized tissue fluid is considerably more alkaline than that of the blood stream since the added increment of extravascular fluid represents only a small percentage of the total fluid in the vascular system, even after full equilibrium has been attained. From the evidence provided by the third samples (c) in Cases 93 and 95 it would appear that this increased alkalinity is of short duration.

DISCUSSION

There is a certain logic which suggests that one of the fundamental changes in toxic women may be a shift of the body generally toward the alkaline side. It has been known for years that during recovery from a severe toxic episode, the urine is definitely alkaline for some days. In the second place, the hyperventilation of normal pregnant women (4) and especially of those suffering from the toxemia of late pregnancy (5), should lead to an acid deficit somewhere in the organism. Finally the eclamptic convulsion which so frequently interrupts the increasingly severe toxemias and is probably to be viewed as a protective phenomenon, constitutes an ideal mechanism for combating an alkalosis. The severe generalized convulsions occurring in a condition of partial anoxemia due to the temporary cessation of respiration inevitably increase the acids in the body as is illustrated by the sudden rise in the blood lactic acid.

The fact that the blood rarely shows a significant change in pH toward the alkaline side at any time during the toxic manifestations has been viewed as opposing the concept of an alkalosis. The data here presented, however, serve to revive the idea that the excess base may be stored in the tissues which generally participate in the physical changes accompanying the disease, and is then released into the blood as the tissue fluids enter the circulatory system to compensate for the blood loss. The increased pH of the second sample in each venesection was definitely

determined by the method of procedure. In spite of the consistent elevation of the pH, the CO_2 capacity and the total acid and base constituents do not vary uniformly.

The explanation for these inconsistencies is not clear. It is apparent that the pH change in the male is the smallest in the series in spite of the fact that more blood was removed from him than from any of the toxic patients. The total acid values do not include the organic acids and it is conceivable that the toxic individuals (even those without convulsions) have more of these substances in the blood, whereas the diluting tissue fluid is relatively deficient. Under such hypothetical conditions the blood would show a decreased total (including the organic) acid content at the end of the venesection. There is no available evidence to support such a contention, even though it would seem to offer the most logical explanation for the invariably increased blood alkalinity at the conclusion of the blood withdrawal.

The lack of uniformity in the behavior of the serum potassium during the blood letting is susceptible of a theoretic explanation. In three of the six toxic women this constituent was slightly increased in two it was definitely decreased and in one there was no change whereas in the male donor it was scarcely altered (a decrease of 0.07 mEq per kgm of water). It is well known that ionic calcium lowers the permeability of a living membrane to potassium ions, and that the major portion of the base in tissue cells is potassium. The two patients who showed decreased serum potassium in the second blood sample were Number 91 a case of cardiovascular renal disease and Number 93, a nonconvulsive toxic patient. In the former it is not unreasonable to postulate some deposit of calcium in the vessel walls or in the surrounding soft tissues, which reduced their permeability to potassium. In the second case the serum calcium was considerably higher in the second blood sample. This finding suggests that the extracellular fluid calcium must have been elevated and that a high tissue calcium might have induced a decreased permeability of the vessel wall and thus have interfered with the passage of the potassium ions into the stream.

CONCLUSIONS

Venesection in women with various forms of the toxemia of late pregnancy induces blood dilution and an associated elevation of the blood pH, which is not accounted for by determined changes in the concentrations of total acid and base

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- 5 Unpublished data

THE EFFECT OF BENZEDRINE (β -PHENYLISOPROPYLAMINE SULPHATE) AND PAREDINE (p -HYDROXY- α -METHYL-PHENYLETHYLAMINE HYDROBROMIDE) ON THE CIRCULATION METABOLISM AND RESPIRATION IN NORMAL MAN¹

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(Received for publication February 5, 1940)

Since benzedrine enjoys wide clinical usage and has definite pressor properties, an evaluation of the effects of its administration on the cardiovascular dynamics is of theoretical and practical interest. Data are available on changes in pulse rate and blood pressure following the administration of benzedrine in man (1, 2); satisfactory studies of changes in cardiac output in man have not, however, been published. It was therefore decided to measure the cardiac output before and after the administration of benzedrine in man, since changes in circulation are intimately related to changes in metabolism and respiratory dynamics; simultaneous studies of the latter were also made.

The marked psychic stimulating effect of benzedrine negates the use of that drug primarily as a pressor substance. Paredrine, closely related to benzedrine chemically, has no stimulating effect on the cerebral cortex but is a potent pressor substance (3). Accordingly, the effects of its administration on metabolism, respiration and circulation were also studied.

The actions of both of these drugs were compared with the effects of the administration of epinephrine, the prototype of the sympathomimetic amines.

MATERIAL AND METHODS

Fifteen subjects ranging in age from 13 to 52 years were used in the present study. 11 were males. No clinical evidence of abnormality of the cardiovascular or respiratory system was present in 14 subjects. 1 subject (Case 13) had partial heart block due to coronary artery sclerosis, but no evidence of congestive failure.

All measurements were made with the patient in the post absorptive state, under basal conditions, in the semi-recumbent position. The minute volume output of the

heart was measured by the method of Starr and Gamble (4); the respiratory rate, respiratory minute volume, tidal air, alveolar carbon dioxide content, respiratory quotient and basal metabolic rate being measured at the same time. The velocity of blood flow was estimated from the arm-to-tongue circulation time, according to the method of Winternitz, Deutsch and Brull (5). Measurements of arterial blood pressure were made by the auscultatory method with a mercury manometer and a standard arm cuff. Pulse and respiration were counted for 30-second periods, every 2 to 4 minutes.

Because of the large number of measurements made on each subject, it was considered desirable to perform all the studies made without the drug on one day and those after the administration of various drugs on other days. It was felt that the effects of increasing restlessness and hunger associated with the performance of protracted experiments might lead to erroneous results. The patients were in the post absorptive state and rested until the pulse rate and blood pressure, as measured at 5-minute intervals, established themselves at constant low levels. The drug to be studied was then administered by mouth or by intramuscular injection. The changes in pulse rate and blood pressure were again measured every 5 minutes until maximal changes occurred. At this point the other studies were begun. Measurements of cardiac and respiratory dynamics were made following the administration of the various drugs only when definite pressor effects were noted, unless the doses used were so small that no pressor effects occurred. In such cases measurements were made at arbitrary intervals after giving the drug. Usually the studies on the effects of drugs were made on successive days after the control measurements. In 2 instances (Cases 4 and 5) however, where the possible cumulative effects of the drug were studied, measurements were made several days after the control studies.

The venous pressure normally may vary as much as 2 or 3 cm. of water from day to day. Since this might be the extent of the change, if any, in this measurement as the result of the administration of a drug, a series of experiments was performed during which measurements of only pulse rate, arterial pressure, and venous pressure were made on the same day immediately before, during and after the administration of a given drug. These are reported in another place.

¹ This investigation was aided by a grant from Smith Kline and French Laboratories, Philadelphia.

CONCLUSIONS

Venesection in women with various forms of the toxemia of late pregnancy induces blood dilution and an associated elevation of the blood pH, which is not accounted for by determined changes in the concentrations of total acid and base

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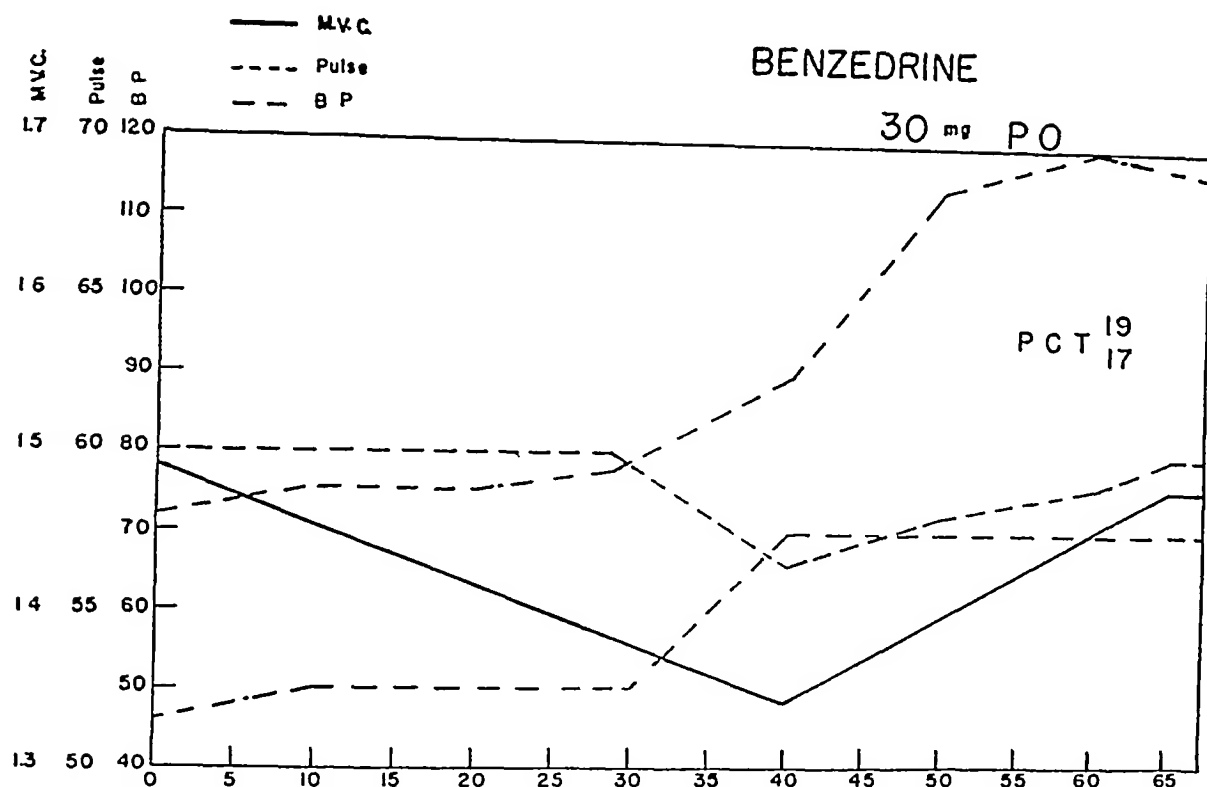


FIG 1 TRANSITORY EARLY DECREASE IN PULSE RATE AND CARDIAC OUTPUT FOLLOWING THE ADMINISTRATION OF BENZEDRINE (CASE 6)

OBSERVATIONS

Cardiac output Benzedrine in doses of 10 to 30 mgm by mouth (Cases 1 to 7, 10, 11, 12) and 10 mgm intramuscularly (Case 8) caused no increase in cardiac output. Paredrine in doses of 30 to 70 mgm by mouth (Cases 11, 12, 13) and 15 to 20 mgm intramuscularly (Cases 14 and 15) likewise caused no increase in cardiac output. These findings are in contrast to those noted after the administration of 1 mgm of epinephrine subcutaneously (Cases 10 and 11), a striking increase in cardiac output occurred after the injection of this drug.

In 2 instances (Cases 6 and 12) an initial decrease in cardiac output occurred after giving paredrine or benzedrine, with normal readings being found a short time later in 1 case (Case 6) (Figure 1).

Velocity of blood flow Acceleration of the circulation time did not occur after the administration of benzedrine or paredrine (Cases 1 to 8, 10, 11, 12, 13, 15). In 1 instance (Case 12) paredrine caused a slight slowing of the circula-

tion time. The injection of adrenalin caused a marked increase in the velocity of blood flow (Cases 10 and 11).

Arterial blood pressure Increases in blood pressure of less than 10 mm were not considered significant. Benzedrine in 10 mgm doses by mouth (Cases 1 to 5) caused a significant rise in blood pressure in only 1 instance (Case 2), but with the same dose given intramuscularly (Cases 8 and 9), or with larger doses by mouth (Cases 6, 7, 10, 11, 12), a definite pressor response was noted. The systolic blood pressure rose more than the diastolic in every instance in which a change occurred, so that the pulse pressure increased. The administration of paredrine (Cases 11 to 15) resulted in similar changes, paredrine is apparently a somewhat more potent pressor drug than benzedrine.

The injection of epinephrine (Cases 10 and 11) resulted in a significant rise in systolic blood pressure but the diastolic blood pressure fell in 1 case (Case 11) and did not change in the other (Case 10).

TABLE I

The effects of benzedrine, paredrine and epinephrine on the circulation, metabolism and respiration in normal man

Case	Oxygen consumption	Basal metabolic rate	Respiratory quotient	Alveolar carbon dioxide concentration	Respiratory rate	Respiratory minute volume	Vital capacity	Cardiac output	Cardiac output per 100 cc. oxygen consumed	Pulse rate	Circulation time	Systolic blood pressure	Diastolic blood pressure	Remarks
	cc. per minute	per cent		per cent	per minute	liters per minute	liters	liters per minute	liters	per minute	sec. or less	mm. Hg	mm. Hg	
1	248 238	+ 5 + 1	0.82 0.80	5.1	15 14	5.8 5.1	2.48 2.50	3.70 3.37	1.49 1.42	63 63	17 18	126 120	78 84	Basal ½ hour after Benzedrine 10 mgm. p.o.
2	258 249	+16 +10	0.81 0.82		14 14	6.5 6.2	2.53 2.52	5.81 5.55	2.25 2.28	98 99	12 14	140 152	100 110	Basal 2 hours after Benzedrine 10 mgm. p.o.
3	255 245	- 4 - 5	0.81 0.81	5.1	13 12	5.7 5.6	4.64 4.58	3.87 3.95	1.53 1.62	74 75	13 14	124 124	82 84	Basal 3 hours after Benzedrine 10 mgm. p.o.
4	167 157	+ 7 -12	0.80 0.79	5.3 5.5	15 12	3.9 2.9	1.90 1.80	2.93 2.93	1.77 1.87	65 83	13 12	108 108	80 84	Basal Benzedrine 10 mgm. p.o. t.i.d. for 2 days 3 hours after last dose
5	223 226	± 0 - 2	0.80 0.79	5.7	14 10	6.4 6.3	3.80 3.90	4.17 4.20	1.87 1.85	76 71	15 16	104 104	80 74	Basal Benzedrine 10 mgm. p.o. t.i.d. for 7 days 1 hour after last dose
6	301 310	+ 9 +12		5.2 5.2	15 14	6.0 6.2	2.60 3.20	5.66 6.00	1.88 1.93	80 85	14 13	128 140	82 96	Basal 1½ hours after Benzedrine 30 mgm. p.o.
7	285 297	- 3 ± 0	0.83 0.80		14 14	8.5 7.8	5.45 5.62	4.43 4.64	1.56 1.56	68 71	12 13	114 150	68 96	Basal 3½ hours after Benzedrine 30 mgm. p.o.
8	242 257	- 4 + 1	0.80 0.78	5.0 5.1	12 13	6.3 5.7	3.20 3.26	4.86 5.14	2.01 2.04	80 93	12 11	106 132	68 80	Basal 1 hour after Benzedrine 10 mgm. i.m.
9	247 242	+ 6 + 3	0.86 0.82	5.5 5.7	11 8	5.7 4.8	3.20 3.30			78 78		106 120	60 70	Basal 1 hour after Benzedrine 10 mgm. i.m.
10	219 222 266	- 7 - 6 +14	0.80 0.83 0.83	5.2 5.3 5.2	13 14 15	4.9 5.1 6.4	4.10 4.50 4.20	4.27 4.57 6.53	1.95 2.06 2.46	55 59 96	13 15 9	104 154 138	60 78 50	Basal 1 hour after Benzedrine 30 mgm. p.o. ½ hour after Epinephrine 1 mgm. s.c.
11	227 265 289 260	+ 1 +19 +28 +16	0.79 0.81 0.79 0.81		16 16 16 16	5.9 6.8 7.2 6.5	2.85 2.90 3.00 2.80	3.37 3.87 5.33 3.66	1.49 1.46 1.85 1.39	58 59 82 48	19 17 12 20	80 132 106 180	60 90 60 90	Basal 1½ hours after Benzedrine 30 mgm. p.o. ½ hour after Epinephrine 1 mgm. s.c. 1 hour after Paredrine 50 mgm. p.o.
12	257 298 256	+ 2 +19 + 2	0.83 0.80 0.80	5.7 4.5 5.5	13 14 14	5.6 8.5 5.7	4.20 4.10 4.10	4.22 4.74 3.56	1.64 1.59 1.39	60 52 46	18 22 22	116 164 160	66 90 86	Basal 1 hour after Benzedrine 30 mgm. p.o. 1½ hours after Paredrine 30 mgm. p.o.
13	171 184	-13 - 8	0.81 0.80	5.4 5.4	11 14	4.0 4.0	1.60 1.60	3.24 3.24	1.91 1.79	40 38	20 19	112 208	50 78	Basal 1 hour after Paredrine 70 mgm. p.o.
14	263 251	+ 9 + 4	0.80 0.80	5.7 5.7	8 9	4.6 4.8	5.20 5.30	5.02 4.81	1.91 1.92	63 63	13 12	120 180	70 88	Basal ½ hour after Paredrine 15 mgm. i.m.
15	256 265	- 2 + 1	0.84 0.81	5.2 5.2	13 14	6.1 6.4	5.10 5.10	5.14 4.82	2.01 1.82	54 48		112 132	74 86	Basal ½ hour after Paredrine 20 mgm. i.m.

Pulse rate The administration of benzedrine caused increases in pulse rate ranging from 4 to 18 beats per minute in 4 experiments (Cases 4, 6, 8, 10) and a decrease of 8 beats per minute in 1 (Case 12) and 5 beats in another (Case 5).

There was no relationship between the dose of the drug given and the change in heart rate. In 2 additional instances (Cases 6 and 9) transitory slowing of the pulse rate occurred soon after benzedrine was given. Paredrine caused a de-

crease in heart rate of 6 to 10 beats in 3 cases (Cases 11, 12, 15), and no change in the other 2. Epinephrine increased the heart rate markedly in both instances in which it was given.

Basal metabolic rate Neither benzedrine nor paredrine affected the basal metabolic rate except in Cases 11 and 12, in both instances the rises in metabolic rate were associated with restlessness. Epinephrine, on the other hand, increased the metabolic rate significantly (Cases 10 and 11). None of the drugs affected the respiratory quotient.

Respiratory dynamics None of the drugs influenced the respiratory dynamics except in those experiments in which the metabolism was raised, in these instances the respiratory minute volumes increased (Cases 10, 11, 12). The vital capacity did not change.

DISCUSSION

Benzedrine, when given in the doses usually employed clinically for its psychic stimulating effect, *i.e.*, 5 to 10 mgm by mouth, exerts little or no pressor effect. In larger doses, however, it causes a definite increase in systolic and diastolic blood pressures. In doses up to 30 mgm given by mouth and 10 mgm intramuscularly, it causes no increase in cardiac output or velocity of blood flow, although the pulse may be increased significantly in some cases. The findings with respect to the output of the heart become even more uniform if these values are related to changes in metabolism. The results of the studies on cardiac output here reported differ from those recorded by Berggren and Soderberg (6) in 2 subjects. These authors concluded that benzedrine increases the cardiac output. Their results, however, are so variable from experiment to experiment, and their control values so abnormal as to suggest some grave technical error in their measurements of the cardiac output. Their findings were not controlled by measurements of the circulation time. The uniformity of the results here reported with respect to cardiac output and circulation time after the administration of benzedrine, and their striking differences from the effects of epinephrine in the same subjects lead us to conclude that benzedrine does not increase the output of the heart. In occasional instances, such as Case 6 (Figure 1), an initial reduction in

cardiac output may be detected. This is associated with the initial slowing of the pulse detected in this and other experiments and is probably due to a reflex initiated by the rise in blood pressure and effected through the vagus nerve. This effect is transitory, however, the cardiac output soon returning to its normal level. The action of benzedrine in causing a reflex stimulation of the vagus nerve may be of importance in precipitating the collapse which occasionally occurs after the administration of overdoses of that drug.

Similarly, in 5 experiments paredrine caused striking elevation of the blood pressure with no increase in cardiac output. These findings are in agreement with those reported by Stead and Kunkel (7) in 2 subjects following the administration of a methyl derivative of paredrine. In 1 instance (Case 12) a decrease in cardiac output and slowing of circulation time due to the action of the vagal reflex were detected. Although 2 other subjects exhibited marked slowing of the pulse, no change in cardiac output was found. It is possible that when the cardiac output is decreased through the action of the vagal reflex, the venous pressure increases and acts to increase the output of the heart to its former level.

The action of benzedrine and paredrine on the normal cardiovascular system differs in several ways from that of epinephrine. The latter causes marked tachycardia, increase in cardiac output and acceleration of circulation time with only moderate transitory elevation of systolic blood pressure and no rise or even a fall in diastolic blood pressure. The observations on the effects of adrenalin recorded here are similar to those previously reported by other authors (8, 9, 10). Benzedrine and paredrine, on the other hand, cause considerable increases in systolic and diastolic blood pressure with no increase in cardiac output or velocity of blood flow. Epinephrine tends to precipitate ventricular arrhythmias, neither paredrine nor benzedrine has been observed to do this.

The increase in pulse pressure which occurs after the administration of benzedrine and paredrine in man does not indicate an increase in cardiac output. The concept that cardiac output parallels pulse pressure, which is widely held, probably dates back to the work of Hürthle (11). However, as long ago as 1904, Erlanger and

Hooker (12) pointed out that theoretically when blood pressure is elevated, the pulse pressure should increase even though the systolic output remains constant vasoconstriction should cause an increase in pulse pressure. The observations of Katz and Wiggers (13) on intact animals have shown that increasing the peripheral resistance raises the blood pressure and increases pulse pressure without changing the cardiac output significantly. Even in the heart lung preparation where vasomotor influences are absent, there is no necessary relation between systolic output and pulse pressure (14).

The effect of the administration of benzedrine on basal metabolism has been studied by others (6, 15, 16, 17) with divergent results. In the experiments here reported, benzedrine caused no change in basal metabolism, in spite of the definite psychic stimulation usually observed unless the subject became restless. It is clear that the administration of benzedrine in the doses usually employed for clinical purposes places no burden on the cardiovascular system, either directly or by raising the metabolic rate.

The prolonged pressor action of benzedrine and paredrine should be of value in clinical conditions associated with fall in blood pressure except that the marked psychic stimulation caused by benzedrine negates the use of that drug. Paredrine having no such action, is the drug of choice in such conditions. Favorable results of its use in orthostatic hypotension (18) and spinal anesthesia (19) have been reported. In addition it has been found in this clinic, to restore the blood pressure to normal in Addison's disease and in shock due to coronary thrombosis. It has also been found a useful adjunct in the treatment of the peripheral vasomotor collapse of hemorrhage, pulmonary embolism and surgical shock. Results in the collapse associated with overwhelming infection have not been satisfactory. It must be borne in mind that the effects of paredrine on the cardiovascular dynamics in all of the above mentioned clinical conditions are not necessarily the same as those observed in this investigation on subjects with normal circulations. Accordingly the widespread use of paredrine in these conditions should await further studies.

SUMMARY AND CONCLUSIONS

1 The effects of benzedrine and paredrine given in doses of 10 to 70 mgm on the metabolism, respiration and circulation were studied in 15 subjects with normal cardiovascular systems. The drugs were given by mouth or intramuscularly. In 2 cases the effect of these drugs was compared to that of epinephrine.

2 Benzedrine and paredrine in doses of 20 mgm or more caused a marked rise in systolic and diastolic blood pressures in normal man. The cardiac output, pulmonary circulation time, vital capacity, basal metabolic rate and respiratory dynamics were not changed.

3 In several instances transitory slowing of the pulse occurred at the onset of the rise in arterial pressure due apparently to a vagal reflex. In some such cases a transitory slight decrease in cardiac output was also detected.

4 The effects of adrenalin in man were quite different from those of benzedrine and paredrine. They consisted in a slight rise in systolic pressure, no change or a fall in diastolic pressure, marked increase in cardiac output and shortening of the circulation time.

5 Benzedrine in doses ordinarily used clinically, i.e. 5 to 10 mgm, has no significant effect on the cardiovascular dynamics.

6 The prolonged pressor action with no increase of cardiac output and no psychic stimulating effect, suggests that paredrine may be a useful drug in the treatment of certain types of vascular collapse especially where stimulation of the myocardium may be undesirable.

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THE EFFECT OF PAREDRIE ON THE VENOUS SYSTEM¹

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In the preceding paper (1), the effect of the number of authors have associated the pressor administration of paredrine on the cardiac output action of paredrine and related drugs with con-

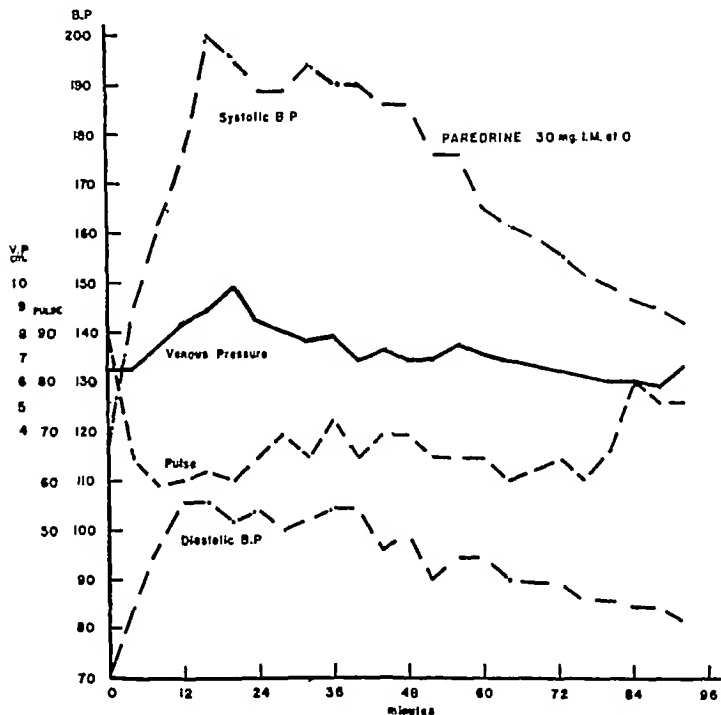


FIG. 1 INCREASE OF ANTECUBITAL VENOUS PRESSURE AFTER THE ADMINISTRATION OF PAREDRIE INTRAMUSCULARLY

and blood pressure of normal man was described. Marked elevation of systolic and diastolic blood pressure occurred but no change in cardiac output other than occasional slight initial decrease associated with vagal inhibition was noted. A

striction of the veins and "venous depots" (2, 3, 4, 5, 6). Since data bearing on this point are inconclusive, it was considered desirable to investigate the effect of paredrine on the veins.

METHODS

Studies were made of the effect of paredrine on the venous pressure in man when given orally intramuscu-

¹ This investigation was aided by a grant from Smith Kline and French Laboratories Philadelphia.

larly, and intravenously. In two of these experiments, the administration of paredrine was preceded by the injection of 2.5 mgm. of atropine. In two experiments adrenalin was given intravenously in order to compare its action with that of paredrine. The subjects used in these experiments ranged in age from 17 to 50, none showed clinical evidences of abnormal cardiovascular function. All studies were made with the subject in the recumbent position at least 2 hours after the last meal, the drug was not given until pulse, blood pressure, and

the drug was given by mouth. Blood pressure was measured by a second observer in the opposite arm at 2-minute intervals by the auscultatory method, using a standard cuff. Radial pulse rate was counted every 2 minutes. The above described studies established the degree of change in the venous system after the administration of paredrine and to some extent also defined the mechanism of this change. In order to evaluate the importance of changes in the venous system on the genesis of the

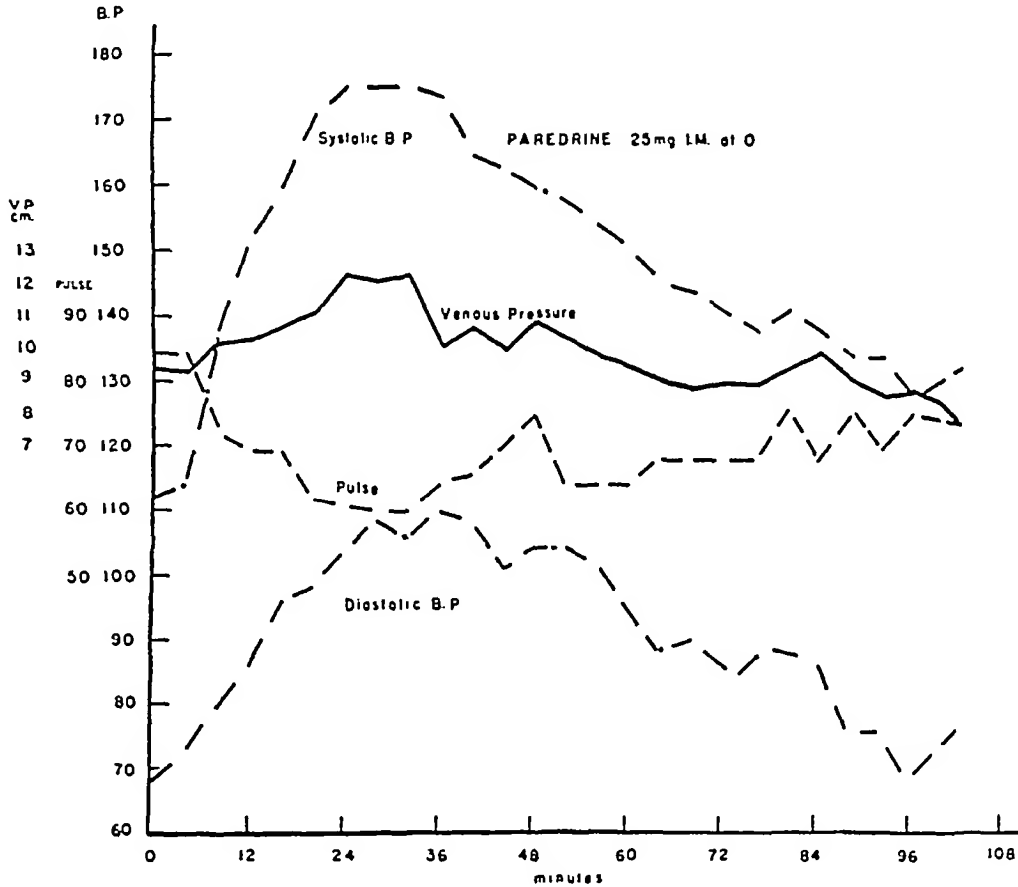


FIG. 2. INCREASE OF ANTECUBITAL VENOUS PRESSURE AFTER THE ADMINISTRATION OF PAREDRIE INTRAMUSCULARLY

venous pressure reached constant low levels. Venous pressure was measured by the direct method (7) in an antecubital vein at 1-minute intervals. In 2 cases simultaneous measurements were made of pressure in the femoral vein at the level of the inguinal ligament. In the studies on adrenalin the drug was injected into a vein of the arm opposite to that used for venous pressure measurements. Since the changes in cardiovascular dynamics produced by oral administration of paredrine are slow in appearing and of long duration venous pressure was determined only initially and at the height of the arterial hypertension in the experiments in which

pressor effect of paredrine, three studies were made on normal men to determine the effect of the inhalation of amyl nitrite on the hypertension caused by the administration of paredrine. Another group of experiments was done on rabbits under nembutal anesthesia. After the injection of paredrine in the ear veins, changes in the veins of the omentum or mesentery of the small intestine of rabbits were determined by measurements of the diameter of the image thrown on a screen by a micro-projector. In a third group of experiments, three in number, studies were made of the rate of flow of solutions of

paredrine through a segment of saphenous vein of anesthetized dogs *in situ* according to the technique of Donegan (8)

RESULTS

Oral intramuscular or intravenous administration of paredrine in man in doses which caused a definite elevation of blood pressure also re-

sure reached its highest level and in all of the experiments the changes in venous pressure were not closely proportional to those of arterial pressure, nor was their time of onset and offset exactly the same. The administration of paredrine produced neither discomfort nor restlessness

Adrenalin (Figure 10) caused a very rapid and

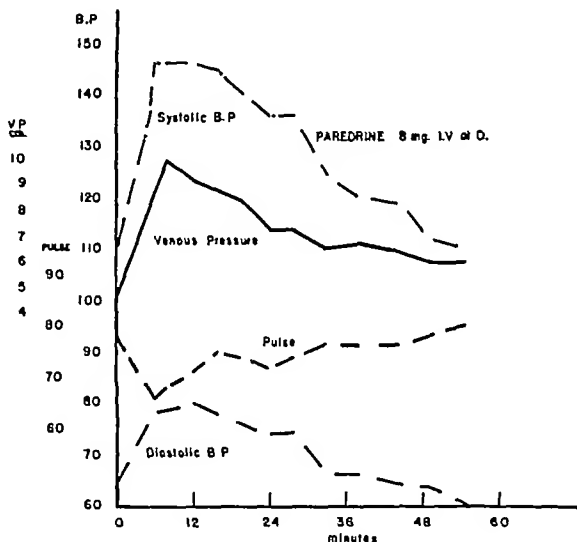


FIG 3 INCREASE OF ANTECUBITAL VENOUS PRESSURE AFTER THE ADMINISTRATION OF PAREDRIENE INTRAVENOUSLY

sulted in a temporary increase in venous pressure of from 2.3 to 6.0 cm. of water (Figures 1 to 7, 11 12). In a few other experiments (not represented in the graphs) no change in venous pressure was found after doses too small to produce increased arterial pressure. The elevation of blood pressure was associated with cardiac slowing except in the atropinized subjects. In these subjects a slight increase in heart rate occurred and increases in both arterial and venous pressure were found to be greater for a given dose of paredrine than in the non atropinized subjects (Figures 8 and 9). In the two experiments in which the drug was given intramuscularly the peak of the venous pressure curve was not reached until several minutes after the systolic blood pres-

sure reached its highest level and in all of the experiments the changes in venous pressure were not closely proportional to those of arterial pressure.

The measurements of the projected diameter of veins of the rabbit showed definite decreases of approximately 20 per cent after the intravenous injection in the ear veins of 5 to 20 mgm of paredrine. In the perfusion experiments on dogs the rate of flow through the venous segment dropped sharply when dilutions of paredrine of 20 mgm per 100 cc. of physiological saline were used. However no definite effect could be obtained by perfusions with dilutions within the range which the usual dosage could produce in the blood stream of an intact animal receiving the drug parenterally.

Inhalation of amyl nitrite (Figures 11 12) in

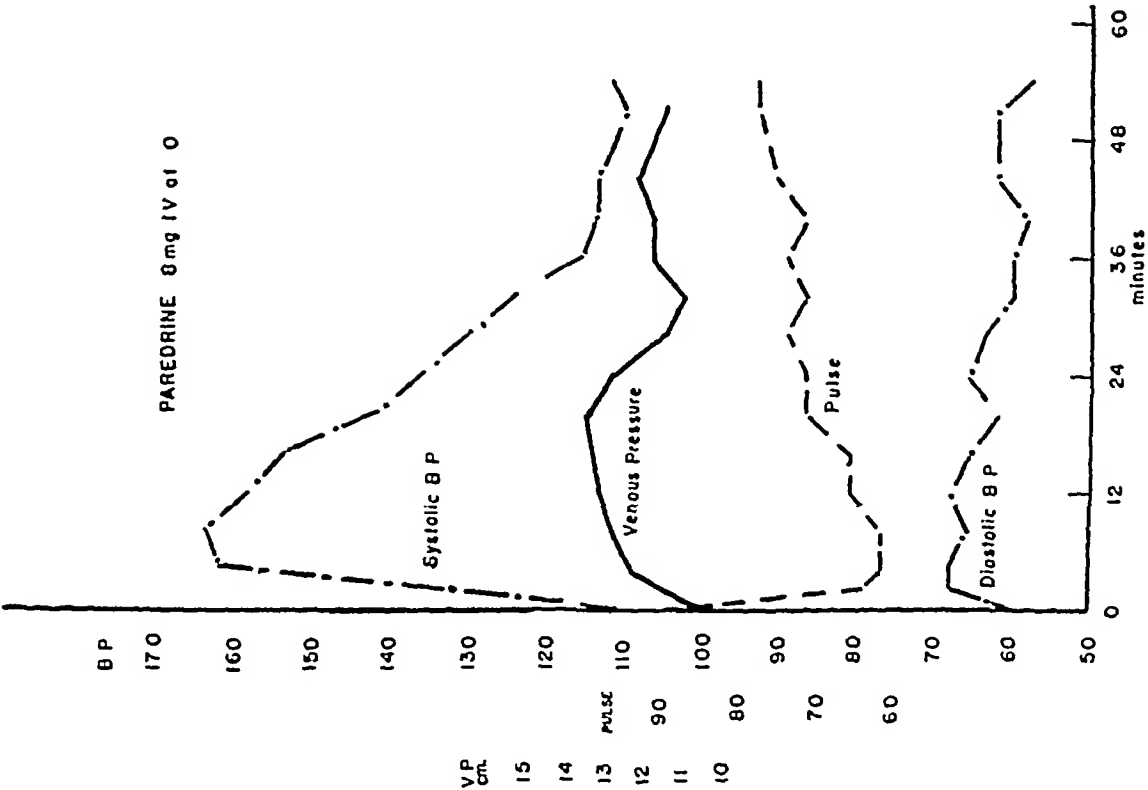


FIG. 4 INCREASE OF ANTECUBITAL VENOUS PRESSURE AFTER THE ADMINISTRATION OF PAREDRIINE INTRAVENOUSLY

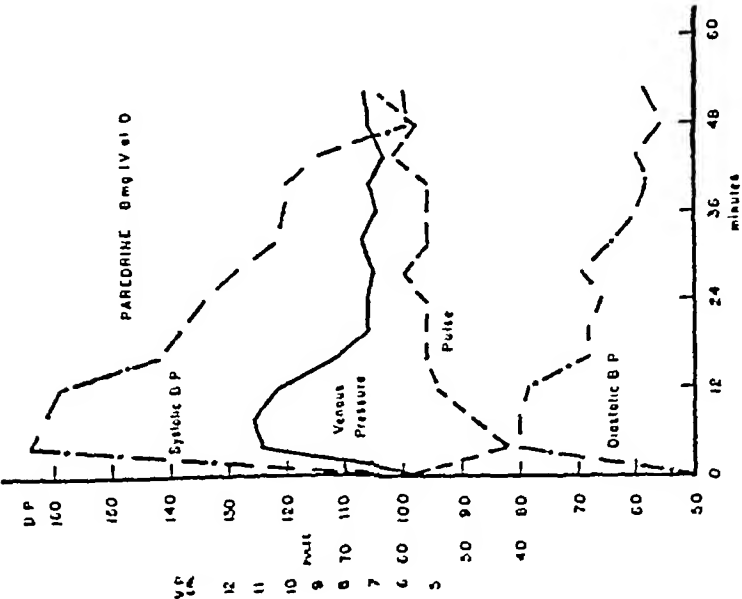


FIG. 5 INCREASE OF ANTECUBITAL VENOUS PRESSURE AFTER THE ADMINISTRATION OF PAREDRIINE INTRAVENOUSLY

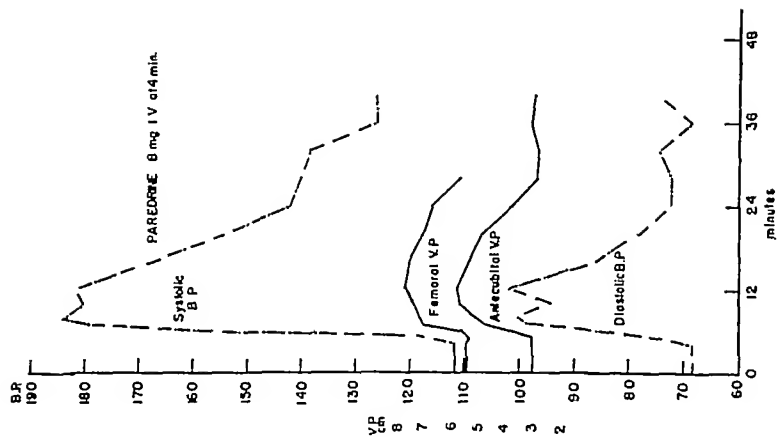


FIG. 6. PARALLEL INCREASE OF FEMORAL AND ANTECUBITAL PRESSURE

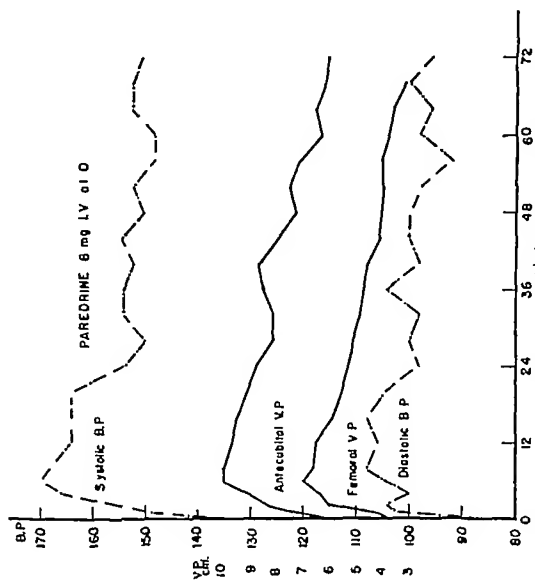


FIG. 7. PARALLEL INCREASE OF FEMORAL AND ANTECUBITAL PRESSURE

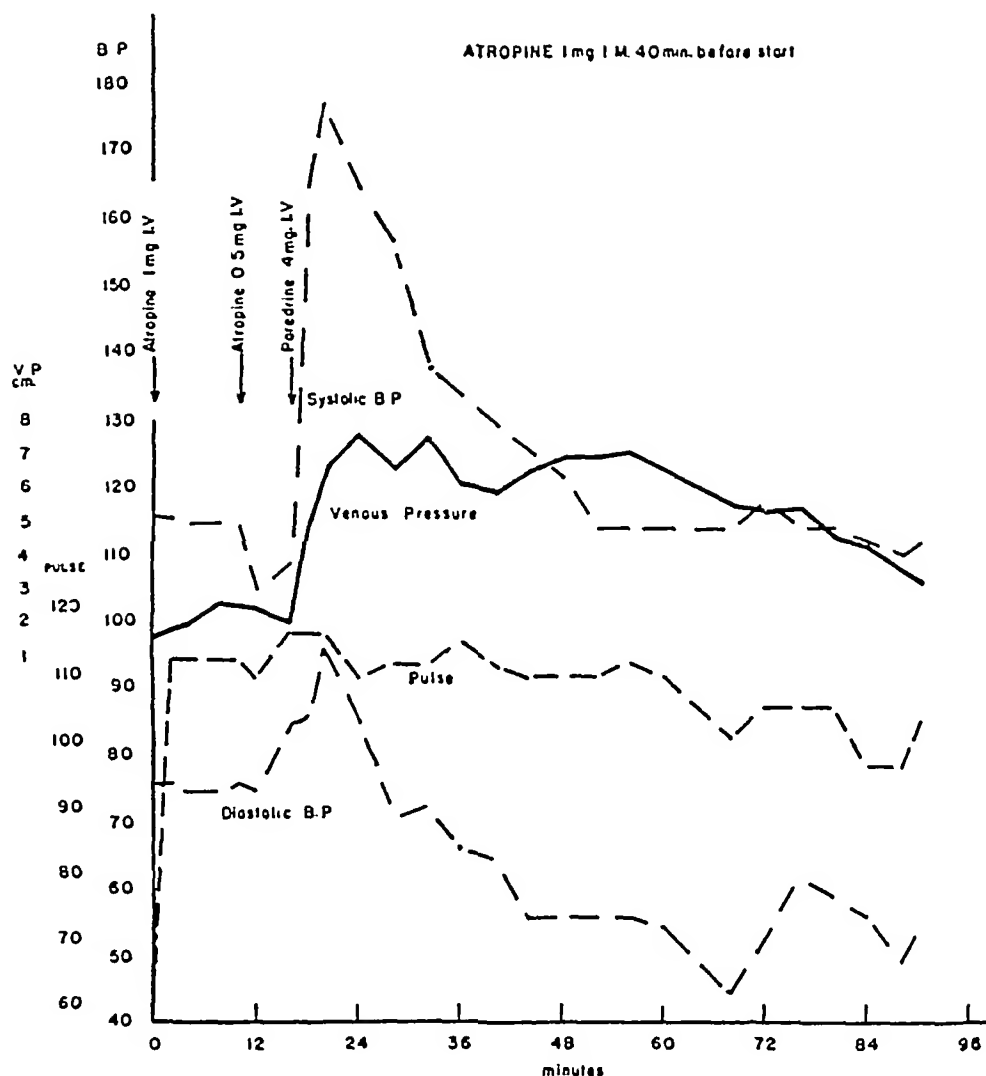


FIG. 8. EFFECT OF THE ADMINISTRATION OF PAREDINE ON ATROPINIZED SUBJECTS

the recumbent posture caused a fall of blood pressure to or below normal levels within 30 seconds, and a return to the hypertensive level within 1 minute. The diastolic levels fell relatively more than the systolic. The experiment was repeated three or four times over a 10-minute interval in each subject, with practically identical results. Marked flushing occurred during the inhalation of the drug. Changes in venous pressure were small.

DISCUSSION

The rise in venous pressure observed after the administrations of paredrine in man is similar to that found by authors who have studied other

sympathomimetic drugs (2, 3, 6, 9, 10, 11, 12, 13). The mechanism causing this phenomenon associated with the administration of sympathicomimetic drugs is not definitely established, some workers favor constriction of the veins themselves (2, 3, 6, 12, 13), while others regard back-pressure from the heart as the cause (10, 11).

Mechanisms which cause rise in venous pressure and deserve consideration here are (a) increase in intrapleural pressure, (b) changes in tone of the skeletal muscle, (c) transmission of the increased arterial pressure through the capillaries into the veins, (d) back pressure resulting from decreased cardiac output due to the vagal slowing of the heart after the administration of

paredrine (c) constriction of the veins, and (f) back pressure due to arterial hypertension.

The first two can be eliminated as causes of the rise in venous pressure due to the administration of paredrine, since the drug causes no change in respiratory dynamics or in the tonus

tion of paredrine is additional evidence that transmission of pressure in this manner does not occur.

In the previous study on the action of paredrine in man (1) it was pointed out that slowing of the heart rate presumably due to depressor reflex almost always is associated with the onset of

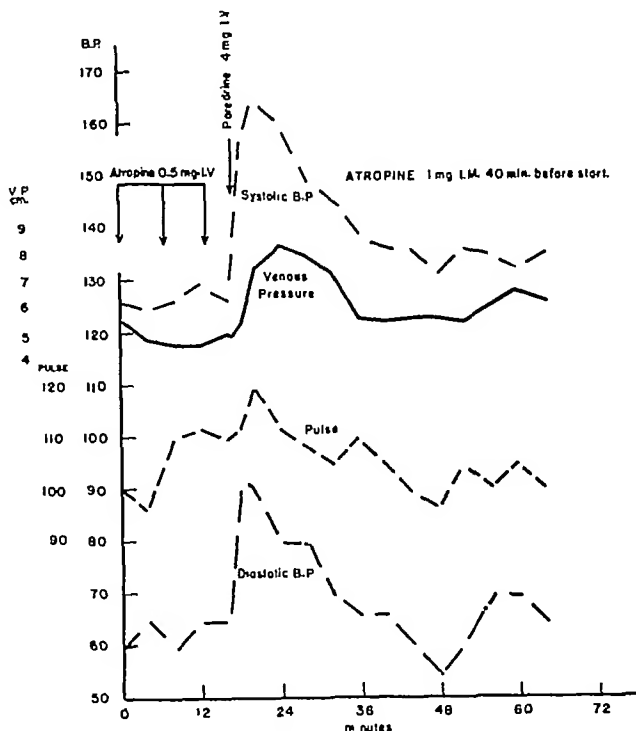


FIG. 9 EFFECT OF THE ADMINISTRATION OF PAREDRIENE ON ATROPINIZED SUBJECTS

of the skeletal musculature. Transmission of increased arterial pressures through the capillaries into the veins is also unlikely since the elevation in blood pressure caused by paredrine is due to arteriolar spasm (14). Landis (15) has shown that constriction of the arterioles in the frog mesentery due to the administration of adrenalin did not increase the capillary pressure. The absence of venous pulsations after the administra-

tion of this drug. In occasional instances a simultaneous slight decrease in cardiac output may also be detected. This might possibly cause an increase in venous pressure. In case of venous pressure secondary to vagal inhibition of the heart is excluded by the experiments on fully atropinized subjects.

Constriction of the mesenteric veins of the anesthetized rabbit was observed to follow

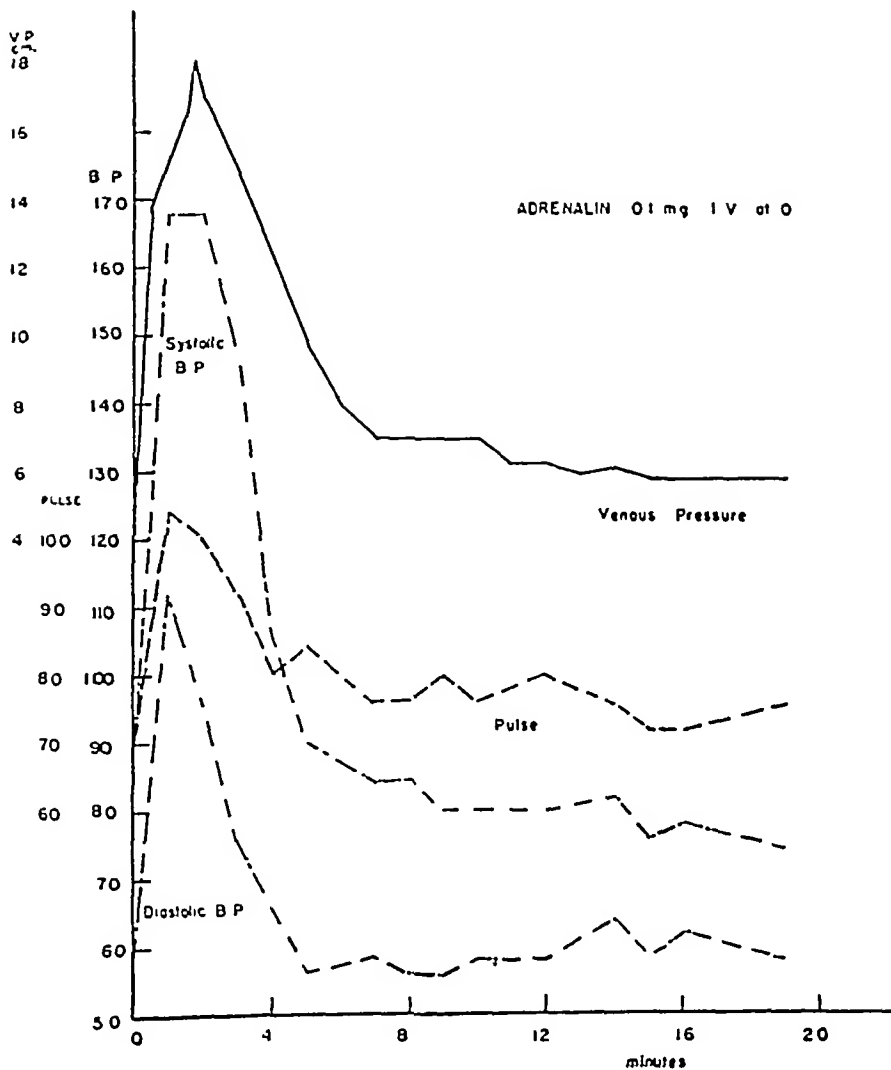


FIG 10 EFFECT OF INTRAVENOUS ADMINISTRATION OF ADRENALIN

injection of paredrine into an ear vein. Studies on the unanesthetized animal were not possible, the only veins available for direct study in such animals are those in the eye-grounds. These, having no muscle in their walls, are incapable of constriction (16), observation of the retina by means of the ophthalmoscope revealed absence of venous constriction, although the retinal arteries were seen to become markedly narrowed. The findings in the studies on the rate of flow of fluid perfused under a constant head of pressure through isolated veins of anesthetized dogs also suggest that paredrine constricts veins, although the unphysiological nature of the animal preparation makes these findings by themselves incon-

clusive. The muscular tone of the vein walls is an important factor in regulation of venous pressure. The resistance of the venous system to central flow of blood is appreciable, as demonstrated by the increase of pressure from the great veins to the capillaries. The discussion of Moritz and von Tabora (7) minimizes the factor of resistance to forward flow of blood in veins, but views the distensibility of venous segments as an important influence on venous pressure. These authors pointed out that if the smooth muscle of the vein wall contracts, a given amount of blood contained within the vein will be under increased tension. Emptying out of the venous "reservoirs" may be considered as a phase of gen-

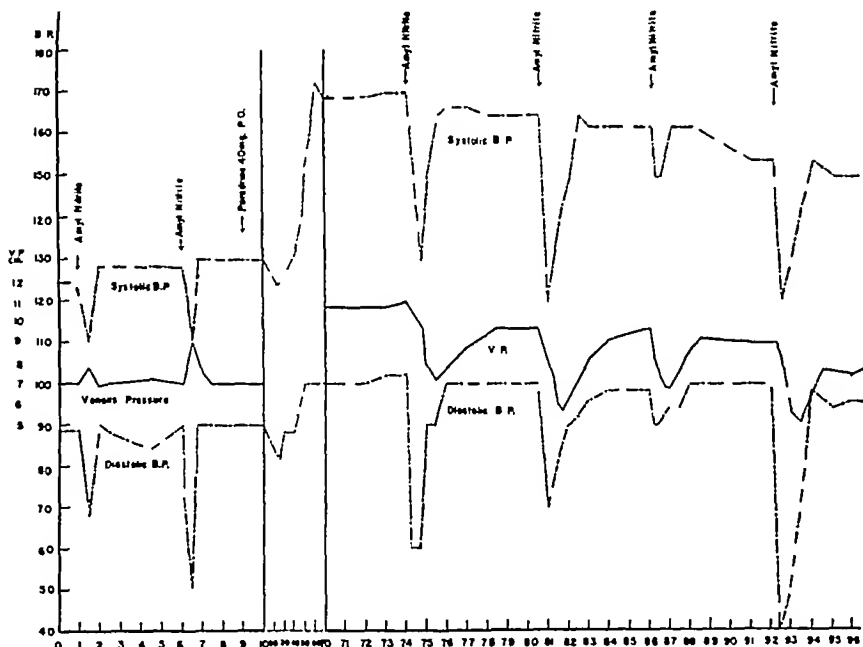


FIG. 11 EFFECT OF INHALATION OF AMYL NITRITE AFTER PAREDRIENE ORALLY

eralized contraction of veins, and might possibly tend to increase venous pressure at least initially in a manner similar to an intravenous infusion of a large volume of fluid (11, 17). It is most probable that venous constriction is one factor responsible for the rise in venous pressure caused by paredrine. This mechanism is not unique to paredrine for the venous pressure increase produced by adrenalin in animals and men has been attributed to venous contraction (9, 12, 13) as has that produced by paredrinol (2, 3, 4, 6).

Measurements of the femoral venous pressure after the administration of paredrine show that the increases in pressure occur in these veins also. These findings indicate that the pressure within the right auricle is also increased, since only large valveless veins lie between the femoral triangle and the heart. The possibility that this increase in venous pressure is due to vagal slowing of the heart is ruled out by the above-de-

scribed experiments on atropinized subjects. The mechanism of the increase in right auricular pressure is not clear. Certain pertinent studies have however been made by other authors. Early experiments with the heart lung preparation (18) showed an elevation of venous pressure if the arterial pressure was raised by increasing the peripheral resistance. Starling considered this to be due to back pressure through the pulmonary circuit. Later workers however attributed this finding either to failure of the heart or to increased filling of the auricle due to increased coronary blood flow (19) which in the heart lung preparation is proportionately much larger than in intact animals. The moderate increases of blood pressure which occur in most of the subjects after the administration of paredrine would not produce cardiac failure. Similarly the absence of decrease of vital capacity rules out back pressure due to cardiac decompensation.

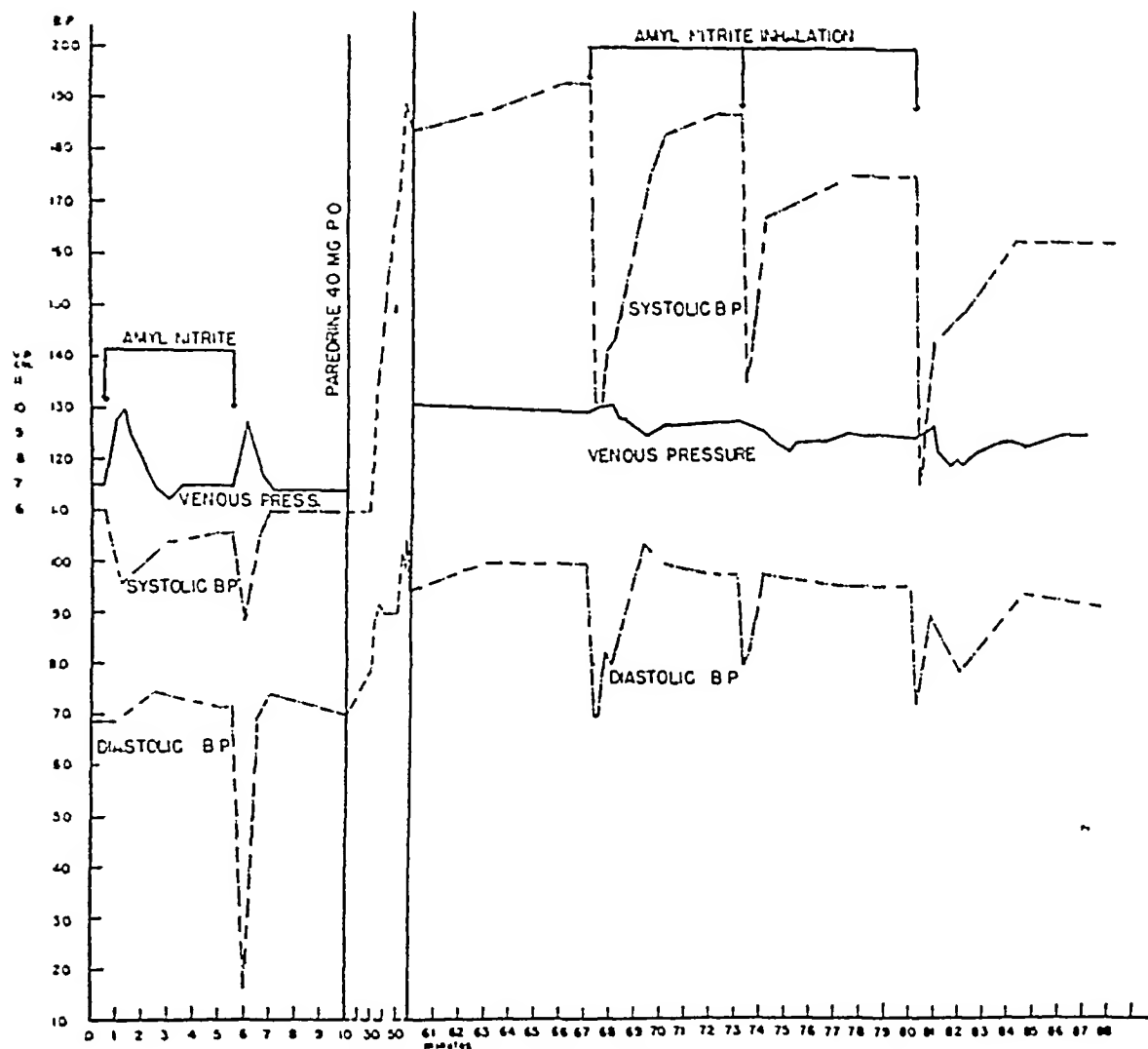


FIG 12. EFFECT OF INHALATION OF AMYL NITRITE AFTER PAREDRIENE ORALLY

The experiments of De Jäger (20), Katz and Wiggers (21) and Barcroft (22) on intact animals showed that obstruction of the aorta close to the diaphragm usually produced increases of venous pressure. These findings are difficult to relate to the present study since the observations of those authors only lasted a few minutes after the occlusion of the aorta, a time during which major changes in the distribution of the blood might be expected to occur. It is quite possible, however, that paredrine produces increased pressure in the pulmonary arterial tree simultaneously with hypertension in the systemic circulation, and this might be suggested as a cause of increased right auricular and venous pressure. However,

in cats, occlusion of as much as 60 per cent of the cross sectional area of the pulmonary artery does not change pressure in the right auricle (23), the venous pressure remaining unchanged until the cardiac output decreases. Since decrease in cardiac output does not occur after the administration of paredrine, it is clear that this mechanism cannot be invoked to explain the rise in auricular pressure caused by the drug. The rise in auricular pressure caused by paredrine must be related to the generalized increase in tonus of the blood vessels throughout the body.

Another important consideration to be discussed is the relation of the increased venous pressure to the arterial hypertension observed after the

administration of paredrine. Authors who have studied paredrinol (2, 3 4 6) have suggested that this drug produces elevation of arterial pressure by increasing the venous return to the heart due to venous constriction. However, many experiments in men and animals show that the intravenous injection of large amounts of fluid (17 24, 25) produces only slight changes in arterial pressure in fact most authors have found that this procedure results in peripheral vasodilatation. The lack of parallelism between venous and arterial pressure curves in our experiments suggests that their variations are produced by independent mechanisms. Since nitrites are said to act chiefly on the veins (26), the observation that nitrites counteract paredrinol hypertension has been used by Stead and Kunkel (6) as evidence that venous changes cause the increased arterial pressure. The rapid decreases in arterial pressure with prompt recovery following inhalation of amyl nitrite are difficult to interpret except as evidence of rapid dilatation and constriction of arterioles it seems impossible for the veins and 'venous reservoirs' to fill and empty so rapidly. It must be concluded that venous constriction is not important in the genesis of the hypertension produced by paredrine in normal man. These findings, however throw no light on the role of venous constriction in recovery from shock.

The pressure increase in the femoral vein indicates that the pressure at the right auricle is probably increased. The classic observations of Starling (27) and Bainbridge (28) would lead one to anticipate increased cardiac output and tachycardia as a result of this increase. Why these changes do not occur is not clear but the increased pressure load against which the heart works must be an important factor.

CONCLUSIONS

- 1 Oral, intramuscular or intravenous injection of paredrine produces a generalized increase of venous pressure in normal man
- 2 Evidence is presented that this increase is due to active constriction of the veins produced by local stimulation.
- 3 Venous constriction is not a factor in the production of arterial hypertension by paredrine

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OBSERVATIONS ON THE BLOOD IODINE I THE BLOOD IODINE IN HEALTH, IN THYROID AND CARDIORENAL DISEASE AND IN LEUKEMIA

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The total amount of iodine in the circulating blood of a normal human adult usually does not exceed half a milligram. It can be understood, therefore, why early investigators doubted that iodine was a constant component of the blood. It was not until 1923 that a method (1) was introduced that was sufficiently delicate to permit a quantitative study of the blood iodine. Since that time, numerous procedures have been brought forward. These have often differed only slightly from each other but have yielded markedly discordant results. For instance, in a recent compilation (2) of normal blood iodine values as given by different workers a range of 11 to 400 micrograms per cent is found.

In the present report an attempt has been made to establish the normal blood iodine level for males and females in New York City and to investigate the effects of certain disease conditions on this norm.

METHOD

After a study of available methods, that of Trevorrow and Fashena (3, 4) proved the most satisfactory and reliable and was adopted for use in this clinic. A description of the method as used in this laboratory was recently published by Palmer, Leland and Gutman (5). Duplicate samples of 15 cc. of blood were digested in 500 cc. digestion flasks which were later connected directly to the distillation apparatus. For this amount of blood, 30 grams of recrystallized potassium dichromate and 90 cc. of the chromic acid sulfuric acid mixture were used.

Small samples were used with bloods suspected of containing large amounts of iodine, as it was shown (5) that quantitative recoveries above 6 micrograms were not always obtained. According to the amount of blood used the proportions of the reagents were varied as follows:

Blood	Potassium dichromate	Acid mixture
cc.	grams	cc.
15	30	90
10	20	60
5	10	30
1	2	10

When 1 cc. or 5 cc. samples were used, digestion was carried out in a 250 cc. flask and a transfer was then made to the large distillation flask with the aid of 100 cc. of redistilled water.

During the hour's distillation care was taken to employ a strong current of alkali washed air, and to collect the same amount of water in the receiving flask as was added to the digestion mixture before distillation (usually 100 cc.). New lots of reagents were always tested for traces of iodine before using.

Total iodine recoveries using this method with known solutions of potassium iodide have been reported from this laboratory (5). Using sheep's blood to which known amounts of potassium iodide were added, equally satisfactory recoveries were made.

In each case the usual rigorous precautions were taken against contamination of the blood sample both when it was secured and after it had reached the laboratory.

Results were discarded when the discrepancy between the duplicate specimens exceeded 10 per cent. The average value of the duplicate determinations is given throughout the present report.

Normal blood iodine

To establish the normal range of blood iodine values, determinations were made on 20 males and 20 females, mostly medical students or laboratory technicians who were in good health. All were receiving a regular diet and the blood samples were usually obtained after breakfast. In each case care was taken to ensure that there had been no recent intake of sea food that tincture of iodine had not been applied to the skin and that iodized salt was not used.

The results are given in Table I and are expressed graphically in Figure 1. The range of the blood iodine for the 20 normal males was 3.8 to 8.6 micrograms and for the 20 normal females was 3.5 to 10.4 micrograms. The average value for the males was 5.9 micrograms, for the females, 6.8 micrograms, and for the entire group, 6.3 micrograms.

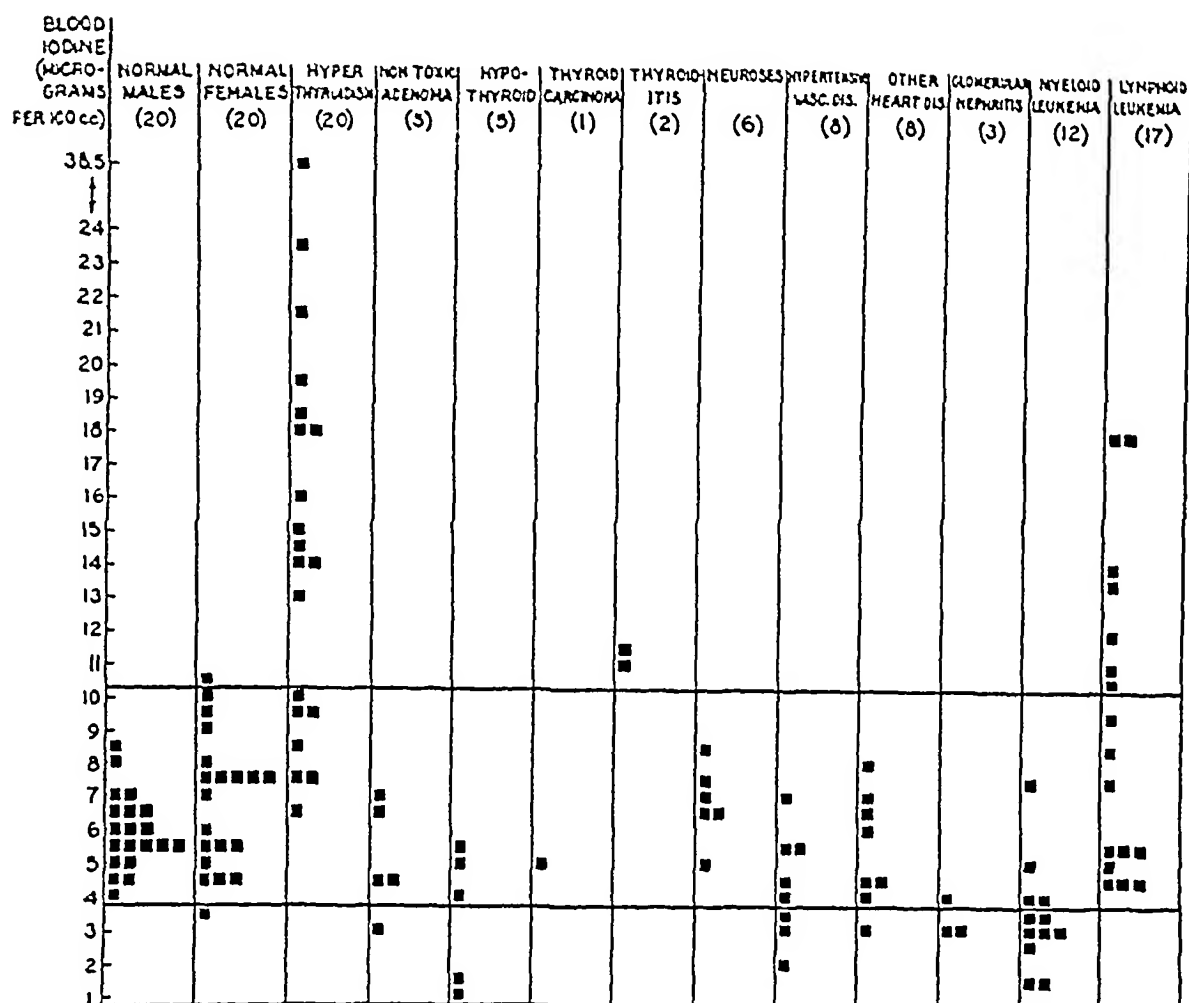


FIG. 1 A COMPARISON OF THE IODINE LEVELS IN THE BLOOD IN HEALTH AND IN THE VARIOUS DISEASES discussed in the text and listed at the top of the columns in the figure. The number of cases in each group is indicated in parenthesis below the diagnosis. Each square represents a patient. The approximate normal range is shown by the heavy horizontal lines. A few minor discrepancies will be noted when the figure is compared with the text due to the necessity of charting according to the nearest half microgram instead of in tenths of a microgram as in the tables.

In general, the results herewith reported for normal adults agree closely with the values given recently for normal infants and children in New York City by Fashena (6), using the same method. She found an average blood iodine of 6.6 micrograms in a group of older children.

It will be noted that the blood iodine varies more widely in normal females than in males and that the average for the females is about a microgram higher. In previously reported series, no constant sex difference in the blood iodine level has been found although it is more often stated that the level is higher in males, contrary to the

results here reported. In all events, the difference, if present, is slight and probably of little significance.

A factor of possible importance that could explain higher values in the female is the generally accepted belief that the blood iodine rises significantly on the first days of the menstrual flow. This was first stated by Veil and Sturm (7), and subsequently without dissent by numerous others. In this series the precaution was taken of securing the blood sample during the intermenstrual period.

With two exceptions all determinations were

TABLE I

Blood iodine of normal adults in New York City

20 Males			20 Females		
Case	Age	Blood iodine micrograms per 100 cc.	Case	Age	Blood iodine micrograms per 100 cc.
139	25	8.6	120	25	10.4
123	27	7.9	19	25	10.2
117	23	7.0	141	23	9.5
34	44	6.8	144	20	9.2
92	37	6.5	136	30	8.1
137	25	6.5	106	22	7.7
91	28	6.5	118	20	7.5
3	26	5.9	138	36	7.4
98	26	5.9	17	29	7.3
18	29	5.8	130	27	7.3
31	26	5.6	143	19	7.0
140	24	5.5	119	25	5.8
107	28	5.5	111	21	5.7
43	34	5.3	121	22	5.6
76	27	5.3	142	25	5.5
63	28	5.2	132	27	5.1
27	25	5.1	112	28	4.6
125	26	4.5	131	26	4.5
110	23	4.3	105	26	4.4
82	28	3.8	124	21	3.5
Average		5.9	Average		6.8

made in the months of September to January inclusive. Within this period no seasonal trend was demonstrable. The average blood iodine values by months follow

Month	Cases	Average blood iodine micrograms
September	9	6.1
October	10	6.6
November	7	5.1
December	3	5.6
January	9	7.5

In previous reports which are too numerous for detailed review here opinion is divided as to the presence of a seasonal effect on the level of the iodine in the blood. When such an effect has been claimed, a rise in the late summer has been noted with the low point occurring in the late winter. We have made 5 determinations of the blood iodine in one normal individual and 9 in a second subject. The results are shown in Fig. 2. From this it is apparent that while there was a considerable variation in the blood iodine level of the individual, no seasonal trend was demonstrated.

As a result of this survey of the blood iodine of normal subjects, it was arbitrarily decided to



FIG. 2. RELATIVELY WIDE FLUCTUATIONS ARE SHOWN IN THE BLOOD IODINE LEVEL OF 2 INDIVIDUALS BUT NO SEASONAL TREND IS EVIDENT

consider the normal range of blood iodine for this method in New York City to be 4.0 to 10.0 micrograms per 100 cc. This decision was accompanied by the mental reservation that perhaps the latter figure was too high and that 9.0 micrograms might be a more accurate upper limit of normal.

Thyroid disease

The intimate relationship of the thyroid to iodine metabolism early attracted the interest of those investigating the iodine content of the blood. As a result, the literature pertaining to the blood iodine in thyroid disease has already become voluminous. A good review has recently appeared (2).

Despite the many previous reports it was considered advisable to study another series of patients with thyroid disorders particularly with the view of using the results obtained as a basis for comparison with other disease groups.

Hyperthyroidism There were 20 cases of undoubted hyperthyroidism (Table II and Figure 1). In 17 the changes in the thyroid were diffuse, and in 3 there was a toxic adenoma. As is usually the case, females predominated in this series in the ratio 3:1. In no instance had iodine therapy been started.

In 14 patients (70 per cent) the blood iodine was above 10.0 micrograms the upper limit of normal. In 2 cases (10 per cent) the blood iodine was 9.5 micrograms, which may be classed as high normal. In the remaining 4 patients (20 per cent) the iodine level was entirely normal. These findings correspond rather closely with previously reported series.

The occurrence of hyperthyroidism with a normal blood iodine level has been repeatedly stressed.

TABLE II
Blood iodine in hyperthyroidism

Case	Sex	Age	Type	Duration	Basal metabolic rate	Serum cholesterol	Blood iodine	Course and remarks
					per cent	mgm per 100 cc	micrograms per 100 cc	
122	M	40	Diffuse	4 months	+48	200	38.5	Well 4 months post-operative
25	F	56	Diffuse	2 years	+40	118	23.7	Well 1 year post operative
20	M	25	Diffuse	1 year	+29	142	21.4	Recurrence in 8 months
					+37	147	13.4	Well 3 months after second operation
93	F	30	Diffuse	1 year	+57	127	19.5	Well 1 year post operative
39	F	60	Adenoma	3 years	+38	98	18.3	Well 3 months post operative
38	F	34	Diffuse	1 year	+49	156	18.0	Well 1 year post-operative
67	F	60	Diffuse	3 years	+23	190	17.8	Well 22 months post operative
77	F	33	Adenoma	6 months	+35	170	16.0	Well 18 months post operative
33	F	39	Diffuse	14 months	+41	175	14.9	Well 15 months post-operative
55	M	25	Diffuse	1 year	+61	129	14.7	Well 2 years post operative
48	M	54	Diffuse	6 months	+47	118	14.2	Improved 14 months post operative
174	M	57	Diffuse	1 year?	+55	159	14.1	Well after 5 months radiotherapy
45	F	49	Adenoma	?	+37	139	12.8	Died
28	F	48	Diffuse	1 year	+75	261	10.2	Well 1 year post operative
14	F	23	Diffuse	2 years	+73	125	9.5	Operated for 3rd recurrence Well 1 year later
60	F	20	Diffuse	9 months	+48	174	9.5	Well 13 months post-operative
85	F	41	Diffuse	4 months	+35	165	8.3	Well 3 months post operative
61	F	24	Diffuse	4 months	+38	208	7.7	No follow up
183	F	30	Diffuse	8 months	+33	145	7.6	Well 4 months post operative
159	F	34	Diffuse	9 years	+18	135	6.7	Operated for 5th recurrence

The present series is too small to warrant detailed comparison between those patients with an elevated blood iodine and those whose blood iodine was normal. Broadly speaking there do not seem to be significant differences between the two groups with two possible exceptions: (1) none of the five males in the series had a normal iodine level, and (2) of the 3 patients observed at the time of a recurrence of hyperthyroidism, 2 had normal blood iodine values. In the third patient, whose blood iodine was 21.4 micrograms when first observed, the level during a recurrence 3 months later was 13.4 micrograms.

No correlation was evident in the entire series between the level of the blood iodine and the basal metabolic rate or the serum cholesterol values determined by the method of Bloor, Pelkan and Allen (8).

Non-toxic adenoma. In 4 of 5 cases of non-toxic adenoma of the thyroid, normal blood iodine levels were found (Table III and Figure 1). In the fifth case the value was low. This patient also had hypertensive vascular disease (see below). Again, as in the hyperthyroid group, there was no correlation of the blood iodine with the basal metabolic rate or serum cholesterol level.

Hypothyroidism. There were 5 cases in whom

the diagnosis of hypothyroidism was established with reasonable certainty. Only 1 presented the clinical syndrome of myxedema. The blood iodine was normal in 2 cases and low in 3 cases (Table III and Figure 1). There was no definite correlation of the blood iodine with the basal metabolic rate or serum cholesterol level.

Thyroiditis. The blood iodine was elevated in 2 cases and the serum cholesterol was low in both, although the basal metabolic rate was low in one and high in the other (Table III and Figure 1). One case was proved by biopsy, in the other the evidence, though presumptive, was strongly suggestive.

Carcinoma of thyroid. In 1 proved case the blood iodine was normal, the basal metabolic rate was elevated, and the serum cholesterol was low (Table III and Figure 1).

"Nervousness." In 6 patients the question of hyperthyroidism had been raised, but after careful study the eventual diagnosis was considered to be some form of nervous imbalance. These individuals were all females and all had normal iodine levels in the blood (Table III and Figure 1). In these cases the basal metabolic rate was normal but the serum cholesterol varied from 140 mgm to 298 mgm per 100 cc.

TABLE III
Blood iodine in miscellaneous thyroid conditions

Case	Sex	Age	Diagnosis	Basal metabolic rate	Serum cholesterol	Blood iodine	Remarks
				per cent	mgm. per 100 cc.	micrograms per 100 cc.	
97	F	38	Non toxic adenoma	- 1	272	7.1	Also rheumatic heart disease
213	F	47	Non toxic adenoma	+ 8	186	6.6	
247	F	53	Non toxic adenoma	- 5	244	4.7	
207	F	78	Non toxic adenoma	+22	156	4.3	Subnormal.
161	F	52	Non toxic adenoma	+10	221	2.8	Also hypertensive disease
64	F	65	Myxedema	-25	355	5.8	
62	F	55	Hypothyroid	-25	278	5.1	
169	M	60	Hypothyroid	- 9	345	3.8	Coronary thrombosis.
21	F	52	Hypothyroid	-17	226	1.7	Recovering from hyperthyroidism induced by thyroid medication
57	F	61	Hypothyroid	-31	420	0.9	Complete thyroidectomy
101	F	39	Carcinoma of thyroid	+35	147	5.0	Febrile. Biopsy diagnosis.
10	F	55	Thyroiditis	-16	124	11.3	Biopsy diagnosis
255	M	47	Thyroiditis	+39	141	11.2	
65	F	34	Nervous ?hyper thyroid	+15	140	8.5	
23	F	35	Nervous ?hyper thyroid	+ 9	224	7.4	
197	F	27	Nervous ?hyper thyroid	+10	241	6.9	
162	F	61	Nervous ?hyper thyroid	+16		6.7	
84	F	35	Nervous ?hyper thyroid	+ 5	298	6.6	
186	F	53	Nervous ?hyper thyroid	+ 2	231	5.2	

Cardiorenal disease

Hypertensive vascular disease All clinicians are familiar with the difficulty sometimes experienced in distinguishing between cases of hyperthyroidism and hypertensive vascular disease. In many instances there is a superficial similarity between the two conditions, but occasionally particularly when the basal metabolic rate is elevated, the differential diagnosis becomes very difficult indeed. It seemed possible that a determination of the blood iodine in conjunction with the serum cholesterol and basal metabolic rate might be of aid in differentiating between them.

No satisfactory information on the blood iodine level in hypertensive vascular disease is available. A number of reports in the German literature indicate a hyperiodemia in this condition (7, 9 to 12), while one investigator (13) reported that a normal level was the rule. When an elevated blood iodine was found the increase did not parallel the rise in basal metabolic rate (11). In the presence of congestive heart failure from whatever cause the blood iodine was reported high (7) or normal (14). Digitalis was observed to produce a sharp

drop in the iodine level (7). In a few cases of renal failure an elevated blood iodine was found (13, 15).

TABLE IV
Blood iodine in hypertensive disease

Case	Sex	Age	Clinical picture	Diagnosis	Non-protein nitrogen	Basal metabolic rate	Serum cholesterol	Blood iodine
					mgm. per 100 cc.	per cent	mgm. per 100 cc.	micrograms per 100 cc.
78	F	51	Impending uremia	+	64	+35	335	6.9
1	M	47	Cardiac failure	+	55	+34	272	5.6
194	F	42	Coronary thrombosis	0			244	5.4
207	F	78	Cardiac failure	+	24	+22	156	4.3
52	F	60	Coronary thrombosis	0	37	- 4	417	4.1
178	F	86	Cardiac failure	+	38	+38	182	3.7
161	F	83	Cardiac failure*	0	40	+10	221	2.8
183	F	49	Headache; retinitis	0	31	+42	393	2.0
Average						+30	278	4.4

* Also had non toxic adenoma of thyroid

The blood iodine was determined in 8 patients with hypertensive vascular disease (Table IV and Figure 1). The only basis for selection was the diagnostic difficulty afforded by all. In each, hyperthyroidism had been considered a possibility

The clinical picture varied. Thus, in 4 patients there was congestive heart failure, in 2 instances there had been a coronary thrombosis, and there was 1 case each of impending uremia and severe headache with retinal changes.

The basal metabolic rate was distinctly elevated in 5 of the 7 patients on whom the test was made. This, of course, is not characteristic of all series of cases of hypertensive vascular disease, and it should again be emphasized that the patients in this particular group all suggested in some way the diagnosis of hyperthyroidism.

The serum cholesterol was high in half the cases.

The blood iodine values are of interest. In 5 patients the iodine level in the blood fell within the lower half of the normal range. In 3 cases there was a hypiodemia. There was no correlation between the blood iodine level and the clinical picture presented by the patient, the basal metabolic rate, or the serum cholesterol. Digitalis had been given to 4 patients but, so far as could be inferred, it had had no particular effect in lowering the blood iodine. The 2 patients in this group with the highest blood iodine values had received digitalis, the 2 patients with the lowest levels had not been given the drug. None of the cases had received quinidine or potassium iodide. In one instance (Number 52) some bromide had been given.

It should be noted that the average patient in this group with hypertensive vascular disease had a basal metabolic rate of +30 per cent, a serum cholesterol slightly above normal at 278 mgm., and a blood iodine of 4.4 micrograms which is at the lower limit of normal.

Other heart disease. For comparison, a group of 8 patients with rheumatic, syphilitic, or arteriosclerotic heart disease was studied (Table V and Figure 1). Six of the patients had congestive heart failure and 2 had aneurysms. Digitalis had been given to 5 cases.

The basal metabolic rate had been determined in only 4 of the group. It was normal in each instance and the average of the 4 determinations was -1 per cent.

In contrast to the hypertensive group, the serum cholesterol was above normal in only 1 case. The average serum cholesterol was 200 mgm. as com-

TABLE V
Blood iodine in miscellaneous cases of heart disease

Case	Sex	Age	Etiology	Clinical picture	Digitalis	Non-protein nitrogen	Basal metabolic rate	Serum cholesterol	Blood iodine
						mgm. per 100 cc	per cent	mgm. per 100 cc	micrograms per 100 cc
54	M	32	Syphilitic	Aneurysm	0			213	7.9
97	F	38	Rheumatic	Cardiac failure*	0		-1	272	7.1
213	F	47	Rheumatic	Cardiac failure*	+	33	+8	186	6.6
152	F	52	Syphilitic	Cardiac failure	+	40		204	5.8
260	F	72	Arteriosclerotic	Cardiac failure	+	33		186	4.6
51	F	24	Rheumatic	Cardiac failure	+	36	+12	132	4.5
126	M	29	Syphilitic	Aneurysm	0	29		170	3.9
167	F	38	Rheumatic	Cardiac failure	+	33	+3	210	3.0
						Average	+1	200	5.4

* Also had non toxic thyroid adenoma.

pared to 278 mgm. in the patients with hypertension.

In 6 cases the blood iodine was normal. In 1 patient it was lowered slightly, and in the remaining case there was a definitely low blood iodine. The average for the group was 5.4 micrograms compared with 4.4 micrograms in the hypertensive cases.

TABLE VI
Blood iodine in glomerular nephritis

Case	Sex	Age	Non-protein nitrogen	Basal metabolic rate	Serum cholesterol	Blood iodine	Remarks
			mgm per 100 cc	per cent	mgm per 100 cc	micrograms per 100 cc	
171	F	27	22	-13	189	3.8	Also acute rheumatic fever
104	M	14	35	-14	446	3.0	Acute nephritis
204	F	50	49	-22	890	2.8	Nephrotic stage

Nephritis. Blood iodine determinations were made on 3 patients with glomerular nephritis (Table VI and Figure 1). While this series is too small to permit sweeping conclusions, it is of interest to point out that (1) the basal metabolic rate was low in all, (2) the serum cholesterol was markedly elevated in 2 and normal in the patient with active rheumatic fever, and (3) the blood iodine was uniformly low. These findings are, of course, the same as those seen in hypothyroidism.

Discussion. Although the cases here reported are few and further work is necessary, it seems

probable that the finding of a low or low normal blood iodine associated with normal or elevated serum cholesterol may be of clinical value in ruling out a thyroid component in patients with hypertension and an elevated basal metabolic rate. This is particularly evident when those cases with recent congestive heart failure in the hyperthyroid (Cases 25, 39, 67) and hypertensive (Cases 1, 161, 172, 207) groups are compared

	Basal metabolic rate	Cholesterol	I ₂
	per cent	mgm.	micrograms
Hyperthyroid	+34	135	19.9
Hypertensive	+26	211	4.1

In heart disease other than hypertensive, the blood iodine also tends to be low but this tendency is not as marked as in hypertensive vascular disease and the basal metabolic rate and serum cholesterol are normal

Leukemia

In a preliminary report (16) it was pointed out that, in a group of 17 patients with leukemia it was usually possible to differentiate between the myeloid and lymphoid types on the basis of the blood iodine values. The myeloid group tended to show a low level of iodine in the blood while in the cases of lymphatic leukemia the blood iodine

was either normal or considerably elevated. An additional 12 cases have been studied, and the results confirm the original conclusion. The total group of 29 patients included 12 with myeloid and 17 with lymphoid leukemia

The results obtained on study of the myeloid cases are shown in Table VII. The blood iodine ranged from 1.3 to 7.4 micrograms was below normal in 9 cases (75 per cent) and the average for the group was only 3.4 micrograms. In 3 patients the iodine values were within normal limits. In one of these (Case 149) it was 4.1 micrograms, which is at the lowest limit of normal. The highest value obtained was 7.4 micrograms in Case 16. This patient had myeloid leukemia beyond reasonable doubt, but because he left the hospital shortly after the test was performed the determination could not be repeated. Case 133 who also had a normal blood iodine level was studied at the time of a complete spontaneous remission of the leukemic process when no evidence whatever of leukemia could be found although her local physician reported that he had found a leukemia blood picture several months previous to hospital admission. Six months after discharge an abnormal blood count was again discovered and the diagnosis of myeloid leukemia was confirmed.

The spleen was enlarged in all patients except the one last discussed. All the cases were anemic and had elevated white blood counts with abnormal leukocytes in the stained smear, again with the ex-

TABLE VII
Blood iodine in myeloid leukemia

Case	Sex	Age	Duration of symptoms	Duration of therapy	Red blood cells	White blood cells	Immature white blood cells	Basal metabolic rate	Serum cholesterol	Blood iodine	Interval before death
					millions	thousands	per cent	per cent	mgm. per 100 cc.	micrograms per 100 cc.	
16	M	27	3 years	3 years	2.9	13.0	9		110	7.4	5 months.
133	F	54	?	?	5.4	5.3	0			4.8	Living at 1 year
149	M	57	5 weeks	0	2.1	23.6	9	+33	62	4.1	5 weeks.
75	M	30	6 months	0	3.5	155.0	31	+30	144	3.9	Living at 14 months.
41	M	32	6 months	0	4.1	104.0	17	+31	177	3.4	Not followed.
241	M	59	8 months	?	4.5	85.0	32		147	3.4	Living at 1 month
190	F	51	1 year	0	1.8	90.5	47		202	3.0	6 weeks
164	M	55	1½ months	0	2.5	26.3	73		158	2.8	1 week.
215	F	56	2 months	0	2.9	169.0	38		123	2.8	Living at 3 months
58	F	56	10 weeks	6 weeks	4.4	28.0	9		206	2.5	Living at 4 months
13	F	52	4 years	2 years	3.1	47.5	17	+38	156	1.7	3 months.
73	M	42	5 years	3 years	3.3	114.0	9	+31	159	1.3	Living at 20 months
Average								+33	150	3.4	

TABLE VIII
Blood iodine in lymphoid leukemia

Case	Sex	Age	Duration of symptoms	Duration of the apy	Red blood cells	White blood cells	Lymphocytes	Basal metabolic rate	Serum cholesterol	Blood iodine	Interval before death
					millions	thousands	per cent	per cent	mgm per 100 cc	micrograms per 100 cc	
7	M	56	6 months	0	3.9	43.0	86	+45	220	17.9	3 weeks
81	M	69	6 weeks	0	4.0	13.0	33	+30	72	17.8	Not followed
44	M	64	2 years	?	5.1	59.0	88		340	14.2	7 months
15	F	47	2 years	2 years	1.4	12.0	90	+31	191	13.3	6 months
9	M	56	2 years	1 year	4.6	6.6	68	+67	185	11.8	Living at 8 months
113	F	25	11 months	0	3.4	56.0	95		262	11.2	Living at 1 year
88	M	73	18 months	0	4.6	9.0	55	+42	100	10.5	Living at 18 months
40	M	61	7 months	1 month	3.7	31.0	79		195	9.3	Living at 4 months
5	M	56	6 months	0	4.5	45.0	92		216	8.6	Living at 18 months
148	M	64	?	0	3.4	44.9	87		169	7.7	2 weeks
203	F	75	6 months	6 months	4.3	5.5	18		192	5.7	Living at 5 months
242	M	19	4 months	0	6.9	88.0	75	+27	312	5.6	4 weeks
175	M	28	3 months	2 weeks	1.9	331.0	97	+25	174	5.3	1 month
90	F	63	2 years+	6 months	3.8	38.0	93	+56	280	5.2	6 months
4	M	75	15 months	?	4.9	9.0	70	+19		4.7	Living at 2 years
56	M	55	2½ years	2 years	5.0	19.0	70		375	4.6	Living at 1½ years
53	M	56	7½ years	6 years	3.8	5.0	43	+54	129	4.3	Living at 1½ years
					Average			+40	213	9.3	

ception of the patient in remission. The basal metabolic rate was distinctly elevated ranging from +30 per cent to +38 per cent in the 5 cases in which it was determined. The serum cholesterol tended to be low. It ranged from 62 mgm to 206 mgm, and the average for the group was 150 mgm.

As may be seen in Table VII, there is no relationship between the blood iodine level and the age or sex of the patient, the duration of symptoms, the amount of radiotherapy, the red blood count, the white blood count, the percentage of immature cells, the basal metabolic rate, the serum cholesterol, or the length of life subsequent to the analysis.

The blood iodine determination was repeated in 3 patients. Case 75 was found to have a blood iodine of 3.3 micrograms 14 months after his original examination had shown a level of 3.9 micrograms. Case 13 rose in 2½ months from 1.7 to 3.0 micrograms, and Case 73 was constant at 1.3 and 1.1 micrograms during 5 months.

It should perhaps be noted that Case 164, here included as myeloid leukemia, was classified by the pathologist at autopsy as an example of monocytic leukemia.

The data on the 17 patients with lymphoid leukemia are given in Table VIII. With a few ex-

ceptions the blood count was high and the percentage of lymphocytic cells was increased. The majority of the patients were anemic. With two exceptions (Cases 148, 203) there was palpable lymphadenopathy associated in a majority of instances with splenomegaly.

The basal metabolic rate was determined in 10 cases and was elevated in all. It ranged from +19 per cent to +67 per cent, with an average of +40 per cent.

The serum cholesterol varied widely. Although the average value of 213 mgm is normal, individual determinations ranged from 72 mgm to 375 mgm. If the normal range is assumed to be approximately from 160 to 250 mgm, it will be noted that in 3 cases there was a hypocholesterolemia and in 5 the serum cholesterol was above normal. The latter is of some interest in view of the consistent elevation of the basal metabolism.

The blood iodine also varied over a wide range but, whereas in the myeloid group 75 per cent of the cases had sub-normal levels of iodine in the blood and 25 per cent were normal, in the lymphoid leukemias 59 per cent (10 cases) were normal, 41 per cent (7 cases) were above normal, and not a single case had a hypiodemia. The range of blood iodine values was from 4.3 to 17.9 micrograms, and the average was 9.3 micrograms.

A study of the data presented in Table VIII shows no other apparent difference between those patients with an elevated blood iodine and those with normal values. Again, as in the myeloid group, there is no relationship between the blood iodine level and the age or sex of the patient the duration of symptoms or of therapy the red or white blood cell counts the percentage of lymphocytic cells the basal metabolic rate, the serum cholesterol, or the subsequent duration of life.

Repeated determinations were made on 1 patient (Case 4). Two months after the blood iodine was found to be 47 micrograms, the test was repeated and the iodine found to be unchanged at 46 micrograms. Thirteen months later a third determination showed a rise to 56 micrograms with an associated increase in white blood count to 32 000 from 9 000 cells.

An elevated blood iodine was found in 2 cases of lymphosarcoma. One of these subsequently developed a leukemic blood picture. Five additional cases of lymphosarcoma had blood iodine values well within the normal range.

In 6 cases of Hodgkin's disease the blood iodine was normal in 5 and slightly elevated in 1 (106 micrograms).

Ten cases of neoplasm of various sites with widespread metastasis were studied. A surprisingly wide variation in blood iodine levels was found. In a case of carcinoma of the stomach and one of unknown origin the blood iodine was low (26 and 3.8 micrograms). Normal values were found in 5 cases in which the primary site was the stomach (2 cases), adrenal, cecum and unknown. In the remaining 3 cases the neoplasm originated in the breast, kidney and stomach and a hyperiodemia was demonstrated (150, 200 and 32.2 micrograms).

Discussion As was stated before (16) the cases of leukemia with blood iodine determinations reported in the literature are not more than half a dozen in number. It has been assumed apparently that the level of blood iodine was generally increased in both types of leukemia. Increased blood iodine values have been reported in myeloid leukemia (17, 19, 20) and in the lymphoid type (7, 17, 19). A normal value was found in 1 case of myeloid leukemia (19), and Stevens (21) using a method closely resembling the one

employed in this work, reported a normal blood iodine in 1 case of lymphoid leukemia.

The contrast between the iodine range in the blood of the 12 myeloid cases and the 17 in the lymphoid group is well shown in Figure 1, and on comparison of Tables VII and VIII. In only 2 cases was there any overlapping of the two groups. In both these instances patients with myeloid leukemia had normal blood iodine values. Both have been previously discussed. In 1 no explanation for the discrepancy could be adduced; the other patient was in a spontaneous remission without any evidence of leukemia.

In both groups there was a complete lack of correlation between the blood iodine level and various other clinical features. In both groups there was an increase in the basal metabolic rate. The average was slightly higher in the lymphoid group (+40 per cent as compared with +33 per cent). The serum cholesterol in this group was also higher. The average in lymphoid leukemia was 213 mgm while in the myeloid type it was 150 mgm, an abnormally low figure. The striking difference between the two groups however, was in the figures for the blood iodine. The average for the group of patients with myeloid leukemia was 3.4 micrograms compared with 9.3 micrograms for the lymphoid group.

SUMMARY

1. The blood iodine of 20 normal males living in New York City ranged from 3.8 to 8.6 micrograms with an average of 5.9 micrograms. Twenty normal females had blood iodine values varying from 3.5 to 10.4 micrograms and averaging 6.8 micrograms. Although a considerable variation occurred in the blood iodine level of 2 individuals no seasonal trend could be demonstrated.

2. The normal range for the blood iodine was arbitrarily set at 4.0 to 10.0 micrograms.

3. In 20 cases of hyperthyroidism the blood iodine was elevated in 14 (70 per cent), normal in 6 (30 per cent). In 4 of 5 cases of non-toxic adenoma the blood iodine was normal while in the fifth patient it was low. In 5 cases of hypothyroidism the blood iodine was low, normal or low. The iodine level in the blood was elevated in 2 cases of thyroiditis, normal in 1 case of car-

circula of the thyroid, and normal in 6 neurotic females. There was no correlation between the blood iodine level and the serum cholesterol or basal metabolic rate within each of these groups.

4 In hypertensive vascular disease the blood iodine was low or low normal and the serum cholesterol was normal or even high when the basal metabolic rate was increased. It was suggested that these findings would aid in differentiating hypertensive vascular disease from hyperthyroidism. In heart disease other than hypertensive the blood iodine tended to be low, but the serum cholesterol and basal metabolic rate were normal.

5 In 12 cases of myeloid leukemia the blood iodine was abnormally low in 75 per cent, normal in 25 per cent. The range was from 1.3 to 7.4 micrograms, with an average of 3.4 micrograms. Contrasted to this, in a series 17 patients with lymphoid leukemia, the blood iodine was normal in 59 per cent, high in 41 per cent, and low in not a single instance. The range was from 4.3 to 17.9 micrograms and the average was 9.3 micrograms. With the exception of 2 cases—1 in a spontaneous remission—there was no overlapping of the two groups.

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RENAL FUNCTION IN PATIENTS WITH GOUT¹

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There are several theories of the pathogenesis of urate² accumulation in patients with gout. A disturbance of elimination of this substance by the kidneys is probably the most popular. This has been challenged however in a previous communication from this laboratory (1) since it appears to be inconsistent with certain experimental data. Whether or not this theory is valid it deserves serious consideration because urates as naturally occurring end products of purine metabolism are disposed of by the body largely by excretion through the kidneys. If one pursues the hypothesis to a logical conclusion any defect of elimination might be inherited or it might be an acquired phenomenon following damage to the functioning units of the kidneys.

The accepted theory (2, 3) of renal physiology in man assumes that urates are present in glomerular urine as are other ultrafiltrable substances and reabsorbed in part by the cells lining the tubules. If the kidneys were primarily at fault in the gouty diathesis, accumulation of urate might be attributed either to (a) a reduction of the number of functioning glomeruli or (b) an increased reabsorption by the tubules. Both of these phenomena are partially susceptible of quantitative analysis now that improved methods for the study of kidney function are available.

A presentation and discussion of kidney function data, as determined by five experimental procedures are contained in this communication. These include tests for the excretion of substances normally present in the body such as urea, creatinine, urate, sodium and chloride and for the excretion of foreign substances introduced parenterally such as phenolsulphonphthalein, neo-10pax

and inulin. Studies were made of normal persons and of patients suffering from diseases other than gout as well as of patients with classical gout. The action of drugs used therapeutically in the treatment of acute and chronic gouty arthritis was investigated.

SUBJECTS

Thirty-one persons acted as experimental subjects. Twenty-two were afflicted with gout; six were patients with diseases other than gout; three were normal persons. The patients with gout were selected without regard to extent of disease or duration of symptoms. It was necessary only that the criteria for the diagnosis of gout were satisfied (1, 4, 5) and that the patients consented to hospital admission for study. All except K. H. were males. Their ages varied from 28 to 81 years. Each patient had had two or more attacks of acute arthritis and on two or more occasions the concentration of fasting serum urate had been greater than 6.0 mgm. per 100 cc. Osseous or cartilaginous tophi were suspected from the roentgenogram in thirteen. Subcutaneous tophi were present in seventeen. All the patients seen during an acute attack of arthritis had responded to full doses of colchicine. Renal stones composed largely of urates had been passed by seven. A summary of the clinical data for each patient with gout is given in Table I. Several of the patients have been described in previous communications (1, 6, 7). Identical initials refer to the same patient. Four men and four women, none of whom showed impairment of kidney function, acted as controls. Their ages varied from 20 to 54 years. A normal inulin clearance (greater than 95 cc. per minute) and a concentration of fasting serum urate less than 6.0 mgm. per 100 cc. were observed in each control subject. One woman with advanced Bright's disease and malignant hypertension completed the group of controls.

METHODS

All of the data were collected while the subjects were in the metabolism ward of the Massachusetts General Hospital. The patients with gout were allowed, during the period of observation, a low purine diet containing about 70 grams of protein, 90 grams of fat and 300 grams of carbohydrate. The control subjects consumed the usual hospital diet which was moderately low in purine. The five methods of studying renal function were (a) determination of nonprotein nitrogen of the

¹ This investigation was supported in part by a grant from the Corn Industries Research Foundation.

² The term urate is preferable to uric acid in a consideration of acid base equilibrium of biological media just as chloride is preferable to hydrochloric acid and phosphate is preferable to phosphoric acid.

TABLE I

Summary of clinical observations on twenty-two patients with gout

Patient	Duration of symptoms	Severity of disease	Serum urate	Subcutaneous tophi	Osteous tophi by x ray	Renal urate stones
	years		mgm per 100 cc			
E Dw	3	Minimal	10.0			
S Co	3	Minimal	11.9			+
F To	1	Minimal	9.8			
H Wa	30	Extensive	6.4	+	+	
L Mu	6	Moderate	9.3	+		
J Ce	3	Minimal	8.9			
P Lc	5	Minimal	7.8	+		+
M Co	19	Minimal	9.2	+		
I Co	5	Moderate	11.5	+		+
W G B	27	Extensive	8.3	+	+	+
W Da	44	Extensive	11.7	+	+	
P Fa	12	Moderate	8.7	+	+	+
L Si	12	Minimal	8.2	+		
J Co	6	Minimal	7.9			
F Na	10	Extensive	14.0	+	+	+
J Sm	21	Extensive	9.7	+	+	
J Co	41	Moderate	10.4	+	+	
A Cas	26	Extensive	11.3	+	+	
A Ca	38	Extensive	8.9	+	+	
C Cr	12	Moderate	10.4	+	+	
A De	9	Extensive	9.2	+	+	+
K He	35	Extensive	9.2	+	+	

serum, (b) testing ability to concentrate solids, (c) measuring excretion of phenolsulphonphthalein dye, (d) pyelography after intravenous injection of neo-iopax, and (e) estimation of rate of clearance of inulin, creatinine, urate, sodium and chloride.

Prior to July 1 1938, urine concentration tests were done by a slight modification of the overnight method described by Fishberg (8). We have arbitrarily set 1022 as the lower limit of normal. After this time, except for I Co, S Co, and J Sm, the "37-hour test" (9) was used. Correction of specific gravity for albumin was made. Under these conditions normally functioning kidneys are able to concentrate to a specific gravity greater than 1028. Fractional phenolsulphonphthalein tests were done according to the method described by Chapman and Halsted (10). Intravenous pyelography followed the standard technique.

Clearance tests for inulin, creatinine and urate, and in some patients sodium and chloride were performed simultaneously. The procedure outlined by Shannon and Smith (11) was followed. Inulin was chosen because its excretion is believed to be an accurate measure of glomerular filtration (3). Creatinine data are included although evidence suggests that this substance is excreted by tubules as well as in the glomerular filtrate. The clearance tests are reported as cc. of plasma cleared per minute and are corrected to a standard body surface area of 1.73 square meters. The clearance data represent an average of at least 3 periods. In some patients as many as 9 periods were used. Good agreement between the

successive periods was obtained. The tests were begun while the subjects were in a basal state. No food, fluid or activity was allowed from 7 00 o'clock in the evening before the test until 7 00 o'clock in the morning on the day of the test. During the hour following 7 00 a.m., 2,000 cc. of tap water were ingested.

Inulin (Pfanstiehl and Company) was prepared as a 10 per cent solution by heating in 0.9 per cent sodium chloride in water. Immediately before use it was passed through a Seitz filter (12). Prior to July 1, 1938, 50 grams of inulin were injected intravenously 15 minutes before the collection of urine and blood specimens was started. After that date 16 grams were given 15 minutes before the first collection period and an additional 7 to 9 grams were given by a slow infusion throughout the collection periods. Before June 1, 1938, 10 grams of creatinine were given orally 90 minutes before collection of specimens was begun, after that date 3 grams were given intravenously just prior to the inulin. A few "reactions" occurred. Headache and chills were encountered most frequently. No untoward symptoms persisted longer than 24 hours. It was concluded that no serious hazards accompany this method of parenteral administration of creatinine and inulin.

All urine specimens were collected by a urethral catheter. We have relied on the conclusion of Hayman and associates (13) that bladder washings do not increase significantly the recovery of urine if the technique of catheterization is faulty. The urine collection periods were timed by a stopwatch. They were usually 10 minutes in length. In some of the first tests they varied from 10 to 20 minutes. Three or more consecutive collection periods were made in one morning. A blood sample was taken at the half way mark in each period.

Blood samples for determination of electrolytes were collected under oil. The tubes were centrifuged immediately. The urine samples were diluted to proper volume within 30 minutes after collection. Inulin was estimated from an iron filtrate of plasma (14) by the difference in the concentration of reducing substances before and after acid hydrolysis (15). Creatinine was determined according to the method described by Golin and Wu (16), urate according to the method described by Benedict and Behre (17), sodium according to the method described by Butler and Tuthill (18). Serum chloride was determined according to the method described by Keys (19) and urine chloride according to the method described by Harvey (20).

EXPERIMENTAL RESULTS

Inulin and creatinine clearance and routine clinical tests

The patients with gout (Table II) were divided into 3 groups according to their ability to clear plasma or inulin. Group I, normal kidney function with inulin clearances of 95 cc. or more per minute. Group II, moderate disturbance of kidney,

TABLE II
Renal function observations on patients with post

Patient	Date of clearance tests	Age	Blood pressure	Average urine flow during clearance tests	Ca of plasma cleared per minute. Average of 3 or more periods		Urea clearance (Urea clearance) $\times 100$ (Inulin clearance)	Fraction of glomerular plasma filtration after first 15 minutes	Maximum specific gravity of urine	Serum non-protein nitrogen	Intravenous pyelography interpretation
					Inulin	Creatinine					
GROUP I. NORMAL KIDNEY FUNCTION BY INULIN AND CREATININE CLEARANCE TESTS											
E.D.W.	April 22, 1933	46	140/90	8.1	125	6.1	4.7	51.3	1.024	25	Normal
E.D.W.	May 20, 1933	46	140/90	12.0	130	6.1	4.7	51.3	1.024	25	Normal
P.T.C.	December 12, 1933	52	140/90	7.5	125	7.1	6.7	50.7	1.017	30	Normal, small kidneys
H.W.A.	March 22, 1933	56	160/90	15.9	123	8.5	9.3	50.7	1.014	30	Normal
L.M.A.	May 7, 1933	28	130/80	17.8	125	8.1	8.1	51.9	1.014	25	Normal
P.C.	May 22, 1933	41	130/70	11.4	130	7.1	6.0	52.5	1.015	25	Normal
P.C.	May 22, 1933	41	130/70	11.4	130	7.1	6.0	52.5	1.015	25	Normal
M.C.O.	May 10, 1934	40	140/90	6.7	145	6.5	6.9	53.0	1.023	25	Normal
J.C.	December 1, 1934	35	134/78	8.7	122	8.5	8.1	50.9	1.023	25	Normal
GROUP II. MODERATE REDUCTION OF KIDNEY FUNCTION											
W.O.R.	March 1, 1933	73	150/88	5.5	88	6.3	11.2	58.5	1.016	25	Calves in bed
W.O.R.	March 27, 1933	73	150/88	5.5	88	6.3	11.2	58.5	1.016	25	Calves in bed
P.F.	June 17, 1933	64	150/70	15.3	72	6.8	13.2	62.3	1.016	32	Calves in bed
L.R.	December 9, 1933	63	150/70	8.3	71	6.9	8.7	61.3	1.016	32	Calves in bed
J.C.	June 31, 1933	64	145/90	14.3	69	8.2	11.9	63.1	1.010	30	Calves in bed
J.R.	January 31, 1934	71	155/78	7.9	65	7.2	10.2	59.7	1.008	28	Calves in bed
J.C.	January 31, 1934	71	155/78	7.9	65	7.2	10.2	59.7	1.008	28	Calves in bed
J.C.	June 28, 1934	81	180/105	5.6	53	4.4	14.8	61.6	1.016	28	Calves in bed
A.C.	November 1, 1934	67	180/105	5.1	40	7.8	13.4	55.5	1.013	22	Calves in bed
GROUP III. SEVERE REDUCTION OF KIDNEY FUNCTION											
C.C.	March 6, 1935	72	155/78	6.6	31	4.3	37.0	73.0	1.012	45	Left kidney not visualized
C.C.	May 12, 1935	73	164/714	3.4	20	4.0	15.3	81.5	1.008	60	Kidney small, excretion slow
C.C.	November 22, 1935	68	170/70	6.0	25	3.5	37.2	62.5	1.013	44	Bleeding of right pelvis
C.C.	October 30, 1935	49	170/100	4.7	12	3.4	33.6	54.3	1.012	70	Significant excretion of dye in 3 hours by left kidney. Both kidneys are severely atrophic

The blood pressure is believed to be normal although the original observations are lost

function with inulin clearances between 55 and 75 cc per minute, and Group III, severe disturbance of renal function with inulin clearances below 45 cc per minute. In most tests a ratio of approximately 1.13 was observed between inulin and creatinine clearances.

There were nine patients in Group I. Their ages varied from 28 to 59 years. All except H Wa had a systolic blood pressure below 150 and a diastolic pressure below 100 mm Hg. The blood pressure of H Wa was 160/90. Four patients only, E Dw, S Co, M Co, and I Co, were able to excrete a urine with a specific gravity of 1.022 or greater in the overnight test. F To was unable to reach the minimum normal level of 1.029 in the "37-hour test". All except L Mu excreted more than 25 per cent phenolsulphonphthalein dye within 15 minutes after injection. The serum nonprotein nitrogen was less than 35 mgm per 100 cc in all patients. Intravenous pyelography was performed in all except E Dw. The excretion of neo-iopax was prompt in each instance. Patient H Wa showed kidney shadows which were believed to be smaller than normal. According to his history he probably had had a mild attack of Bright's disease (? acute glomerular nephritis) as a young man. P Le had had a urate stone removed from the left kidney pelvis one month before admission to the metabolism ward. The finding of an indistinct pelvis on the left has been attributed to trauma at operation and subsequent diminution in capacity. Thus, while all patients in this group showed normal kidney function by the inulin and creatinine clearance tests, only four showed normal function by all of the tests. Inability to concentrate urine maximally appears to be the first indication of renal impairment in patients with gout.

There were nine patients in Group II. Their ages varied from 31 to 81 years. Five patients, W G B, P Fa, L Si, F Na, and J Sm, had a normal blood pressure. Two of the remaining four, J Go, and A Cas, with slight and moderate elevation of systolic pressure, respectively, had had attacks of hypertensive encephalopathy and elevation of systolic blood pressure over 200 mm Hg. All patients showed an inability to concentrate urine maximally. The specific gravities ranged from 1.018 to 1.009. Only three patients, P Fa, J Go, and F Na, were able to

excrete more than 25 per cent phenolsulphonphthalein in 15 minutes. The concentration of non-protein nitrogen in the serum was within normal limits in all. Neo-iopax was injected intravenously in each patient except W G B. Only two pyelograms, those of F Na and J Sm were interpreted as normal. P Fa showed prompt excretion of dye but the right kidney was small and shadows suggestive of stones were visible bilaterally in the calices. W Du, L Si, and A Cas exhibited indistinct or incomplete filling of the calices, and J Go indistinct kidney pelvis. J Co showed a slight delay in the excretion of the dye.

There were four patients in Group III. Profound impairment of renal function was present in all. The ages varied from 42 to 72 years. Three of the four showed an elevation of systolic or diastolic blood pressure. The specific gravities in the concentration tests varied between 1.013 and 1.008. The excretion of phenolsulphonphthalein dye in 15 minutes was 6 per cent or less. The concentration of serum nonprotein nitrogen varied between 45 and 70 mgm per 100 cc. Following the injection of neo-iopax in A Ca the left kidney was not visualized, the right kidney excreted the dye promptly. C Cr had small kidneys bilaterally, on the right there was dilatation and blunting of the kidney pelvis. The pyelogram of A De, taken after right-sided nephrectomy, showed a small amount of dye excreted by the left kidney at the end of 2 hours. K He had small kidneys with slow excretion of dye. All of the patients had an albuminuria which varied from one plus to three plus.

Clearance studies on the controls are given in Table III. Eight exhibited normal renal function, i.e., the clearance of inulin was greater than 95 cc per minute. H Mc, suffering from advanced Bright's disease, had an inulin clearance of 8.5 cc per minute.

Urate clearance

If urates are filtered completely by the glomerulus (3, 21, 22) and reabsorbed partially by the tubules (23), then the percentage of filtered urate appearing in bladder urine is measured by the ratio $\left(\frac{\text{urate clearance}}{\text{inulin clearance}} \right) \times 100$. Likewise,

TABLE III
Clearance studies on patients without gout

Patient	Sex	Age	Cc. of plasma cleared per minute. Average of 3 periods					$\left(\frac{\text{Urate clearance}}{\text{Inulin clearance}}\right) \times 100$	$\left(1 - \frac{\text{Urate clearance}}{\text{Inulin clearance}}\right) \times 100$	Diagnosis
			Inulin	Creatinine	Urate	Sodium	Chloride			
									per cent	
NORMAL KIDNEY FUNCTION										
L.B.	F	22	148	190	10.5	2.75	5.13	7.5	23.5	Hyperparathyroidism, renal stones
B.D.	F	29	127	144	18.9	2.09	2.90	13.5	87.5	Normal
V.M.	F	54	118	123	11.8	1.57	2.11	10.0	90.0	Hyperparathyroidism
O.K.	M	25	114	161	12.4	1.80	3.01	10.9	84.1	Epidermolysis bullosa
R.J.	M	30	105	149	12.4	1.83	2.53	11.5	83.3	Normal
G.A.	M	34	98	123	8.5			8.5	01.2	Excessa
F.B.	M	20	97	123	7.8	0.54	1.00	8.1	91.9	Normal
L.G.	F	23	96	143	8.9			9.3	90.7	Latent syphilis
EXTREME IMPAIRMENT OF KIDNEY FUNCTION										
H.Ma.	F	43	8.5	12.5	7.5			88.3	11.7	Malignant hypertension, Bright's disease

$\left(1 - \frac{\text{urate clearance}}{\text{inulin clearance}}\right) \times 100$ expresses the percentage of filtered urate reabsorbed by the tubules. Such observations on the nine gouty subjects in Group I (Table II) and on the eight control subjects in Table III with normal kidney function are similar. In both groups approximately 10 per cent of the urate which crossed the glomerular membrane appeared in the bladder urine, 90 per cent was reabsorbed. It is concluded therefore, that a normal urate clearance is approximately 10 cc. per minute.

The gouty patients in Group II showed a moderate reduction in urate as well as in inulin clearance. The averages were 65 cc. and 68 cc. per minute respectively. The reduction in urate clearance appeared to be related to reduction in inulin clearance as the ratio $\left(\frac{\text{urate clearance}}{\text{inulin clearance}}\right) \times 100$ was only slightly greater than that in Group I. It is possible that W. Da. and J. Sm. did not have a diuresis sufficient to produce a maximum excretion of a threshold substance such as urate. The urate clearances in these two patients, therefore, were probably lower than might be produced under better experimental conditions.

The four patients with severe impairment of renal function (Group III) showed an average urate and inulin clearance of 68 and 24 cc. respectively. The average value for the ratio $\left(\frac{\text{urate clearance}}{\text{inulin clearance}}\right) \times 100$ increased to 28 and,

hence tubular reabsorption decreased to 72 per cent. H. Mc. (Table III) was the only control studied who had terminal Bright's disease. The urate clearance on this patient was 75 cc. per minute. More than 88 per cent of the filtered urate was excreted in the urine and less than 12 per cent was reabsorbed.

The relationship between inulin clearance and percentage tubular reabsorption of urate is shown in Figure 1. It is apparent that progressive renal damage in gouty as well as in non gouty patients is associated with an increase in urate excretion relative to glomerular filtration. The percentage reabsorption of urates by the tubules is not depressed however until the inulin clearance is decreased below 50 cc. per minute or approximately one-half of the normal rate. Only when glomerular filtration is reduced to a negligible amount does retention of urate from failure of renal excretion assume pathological significance.

Action of drugs

The effects of cinchophen in therapeutic amounts on the clearance of inulin, urate, sodium and chloride are given in Table IV. Two grams of cinchophen were given orally in divided doses on the day before the clearance tests were done and an additional dose of 0.5 gram each was given at 6:00 and 7:00 on the morning of the test. The clearances of inulin, sodium and chloride were un-

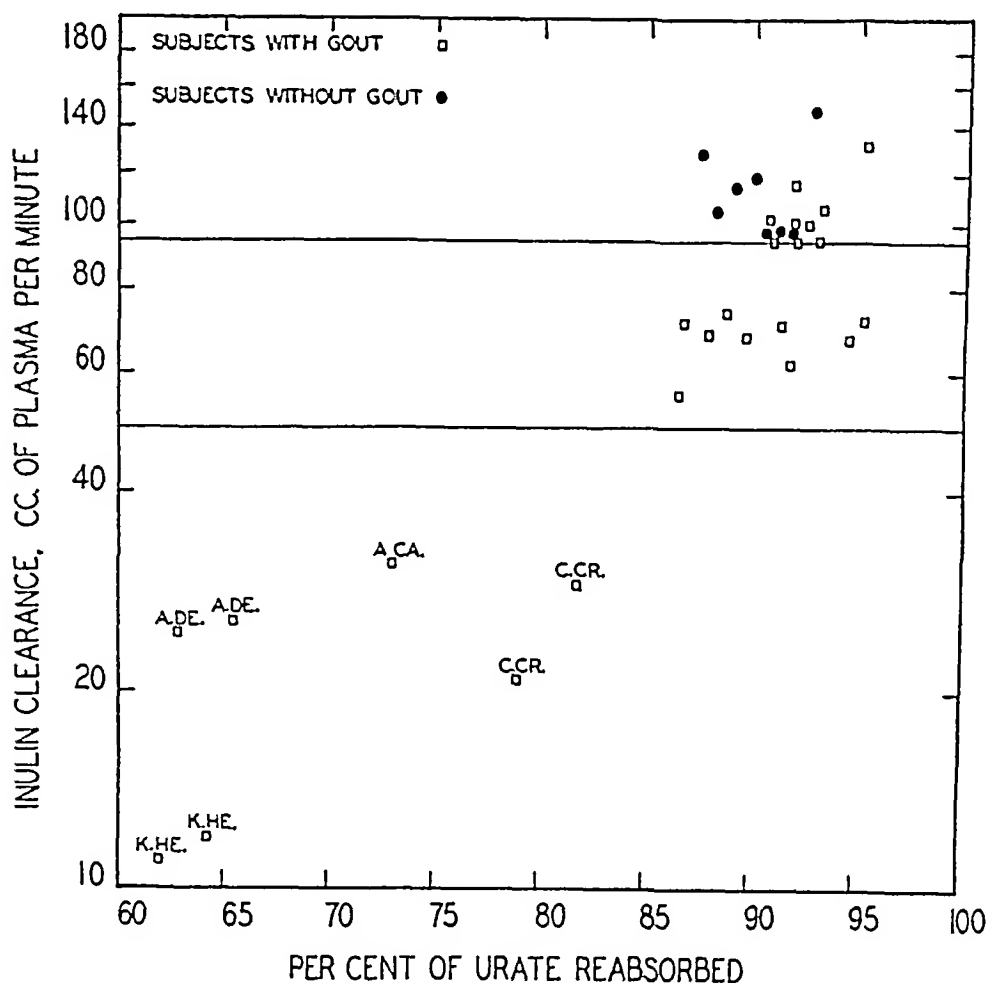


FIG. 1 RELATIONSHIP BETWEEN INULIN CLEARANCE AND PERCENTAGE TUBULAR REABSORPTION OF URATE

changed following the ingestion of this quantity of cinchophen. Urate clearances, however, were increased significantly. Four patients with moderate reduction of renal function, F Na, J Go, L Si, and A Cas, showed urate clearances which were two- or threefold greater than during the control period. R J, one of the non-gouty patients who was given cinchophen, showed a similar increase. The percentage of urate reabsorbed from the glomerular filtrate decreased from 90 per cent without cinchophen to between 62 and 75 per cent with cinchophen. The patients with severe renal damage, A De and C Cr, showed slight increases in urate clearance after ingestion of cinchophen, although the per cent reabsorbed by the tubules was similar to that of the other patients. The serum urate concentration during

each cinchophen experiment was decreased from the value observed during the control experiment.

Salyrgan was given intravenously on two occasions to F Na and on one occasion to J Sm (Table V). Neither patient suffered from acute gout following the administration. An increase in urate clearance was observed in all experiments. The urate reabsorption decreased from approximately 90 per cent to between 30 and 50 per cent. Salyrgan was given to J Sm 3 hours before the clearance tests were started. A satisfactory urine flow was obtained although the diminution in inulin clearance was appreciable. The clearances of urate, sodium, and chloride were increased several fold. In the first experiment on F Na, the clearance data were collected 10 hours after the administration of the drug. A diuresis of 2 liters

TABLE IV
Action of cinchophen

Patient	Date		Serum urate	Cc. of plasma cleared per minute. Average of 3 periods				$(1 - \frac{\text{Urate clearance}}{\text{Inulin clearance}}) \times 100$	Medicine given
				Inulin	Urate	Sodium	Chloride		
			<i>mgm. per 100 cc</i>					<i>per cent</i>	
F Na.	January 16 1939		14.0	68	7.0	1.23	1.90	90.0	
F Na.	January 30 1939		8.8	65	15.7	0.61	1.10	75.8	3 grams cinchophen
J Go	June 21 1938		7.9	69	8.2			88.0	
J Go	June 24 1938		6.0	74	18.0			75.5	3 grams cinchophen
L.Si.	December 9 1938		8.2	71	6.2	0.91	1.60	91.3	
L.Si.	December 15 1938		5.0	74	16.6	1.69	2.50	77.8	3 grams cinchophen
A Cas.	November 11 1938		11.3	56	7.5			86.6	
A Cas.	October 27 1938		6.3	53	20.1			62.2	3 grams cinchophen
A De	November 22 1938		9.2	25	9.3	2.23	3.18	63.0	
A De.	December 2 1938		7.4	28	13.4	3.39	4.90	54.0	3 grams cinchophen
C.Cr	May 12 1938		10.4	29	5.3			82.0	
C.Cr	June 4 1938		7.5	23	6.4			71.7	3 grams cinchophen
R.J	January 4 1939		4.6	105	12.4	1.88	2.83	89.0	
R.J	January 12 1939		2.4	102	29.5	1.43	2.58	71.0	3 grams cinchophen

in excess of the usual output was noted. The urine flow was reduced at the time of clearance studies although the customary amount of water was ingested. The small urine flow and decrease in clearances of inulin, urea, sodium and chloride suggest that certain aspects of the diuresis had passed before the test was started. The second experiment on F Na. was considered the best one to illustrate the relevant tubular effects. Salyrgan was given 3 hours before the tests. Clearances of inulin and urea were similar to the control ex-

periment. Urine flow and clearance of urate, sodium and chloride were increased many fold. These data are difficult to interpret. Salyrgan may precipitate an attack of acute gout (4-24) and no therapeutic claims have been made for it in this malady. Inspection shows however, that its effect on urate clearance is similar to that of cinchophen.

Five patients were given 5 mgm. each of crystalline colchicine (Table VI) in divided doses during a 24-hour period prior to the test. This is an

TABLE V
Action of salyrgan

Patient	Date		Diuresis	Serum urate	Cc. of plasma cleared per minute. Average of 3 periods					$(1 - \frac{\text{Urate clearance}}{\text{Inulin clearance}}) \times 100$	Medicine given
					Inulin	Urea	Urate	Sodium	Chloride		
			<i>cc. per minute</i>	<i>mgm. per 100 cc</i>						<i>per cent</i>	
F.Na.	January 16 1939		9.6	14.0	68	43	7.0	1.23	1.90	90.0	
F.Na.	February 13 1939		5.6	11.7	67	23	7.1	1.58	2.46	89.4	
F.Na.	February 15 1939		3.5	8.8	55	23	37.7	0.33	0.80	31.4	2 cc. salyrgan, 10 p.m., February 14, 1939
F.Na.	February 21 1939	16.3		11.0*	69	46	32.9	12.70	18.00	52.3	2 cc. salyrgan, 7:45 a.m., February 21 1939
J.Sm.	March 24 1939		1.7	9.7	67		3.5			90.0	
J.Sm.	April 3 1939	12.6		7.0	37		25.6	9.05	11.60	30.7	2 cc. salyrgan, 7:45 a.m., April 3 1939

* Serum urate was 14 mgm. per 100 cc. on the previous day

TABLE VI
Action of colchicine

Patient	Date	Cc of plasma cleared per minute Average of 3 periods		$(1 - \frac{\text{Urate clearance}}{\text{Inulin clearance}}) \times 100$	Medicine given
		Inulin	Urate		
A. Cas	November 11, 1938	56	7.5	<i>per cent</i> 86.6	5 mgm colchicine
A. Cas	November 12, 1938	56	8.1	85.6	
J. Co	June 28, 1938	62	5.1	91.8	5 mgm colchicine
J. Co	June 30, 1938	64	6.0	90.6	
P. Fa	June 17, 1938	72	9.5	86.9	5 mgm colchicine
P. Fa	June 19, 1938	80	11.1	86.1	
L. Si	December 9, 1938	71	6.2	91.3	5 mgm colchicine
L. Si	December 13, 1938	68	6.3	90.6	
A. De	November 22, 1938	25	9.3	63.0	5 mgm colchicine
A. De	November 28, 1938	26	9.0	66.0	

average therapeutic amount used by us in the treatment of an acute attack of gouty arthritis and is effective in most patients. No significant change in inulin or urate clearances was observed. It is apparent that the beneficial effect of colchicine is not accompanied by any demonstrable change in urate clearance.

DISCUSSION

This study of renal function on patients with gout confirms many clinical observations (25, 26, 27). The constancy of the results by the 5 methods employed is satisfactory. Eighteen of the twenty-two patients in Table II show some limitation of renal function. The inability to concentrate solids maximally (28) appears to be the first evidence of failure. In Group I, five patients with normal inulin and creatinine clearance rates were unable to concentrate maximally. In Group II, each patient had a lowered specific gravity. All of the patients in Group III showed renal deterioration by each of the tests. As an approximation, it may be stated that neither duration of symptoms of gouty arthritis nor degree of elevation of serum urate are the sole determining factors in producing renal dysfunction. It is noteworthy that the age of the patient appears to be of secondary importance and longevity may not be impaired.

Hypertension and arteriosclerosis have been associated with a reduction in kidney function (29)

in various maladies. It is pertinent, therefore, to consider their incidence in patients with gout. In our series only eight had hypertension, in each of these it corresponded to the benign rather than the malignant type (30). K. H. developed hypertension under observation but not until 3 years after laboratory tests; there severe impairment of renal function. She had recurring cystitis and mild pyelonephritis, factors which probably contributed to the hypertension. A. Ca, on the other hand, also had had cystitis but at no examination was hypertension noted. A. De had bilateral renal stones and a systolic blood pressure of 170 mm Hg. The other patients with hypertension gave no history of urinary tract infection nor did they have any signs or symptoms suggesting it. The development of arteriosclerosis in patients with gout occurs probably earlier than hypertension. All of the patients in Group II, which includes F. Na, a man of 31, had sclerosis of the peripheral arteries. Two of the four in Group III, A. Ca and A. De, showed similar changes. Each of the three who were studied at necropsy, J. Sm, A. Cas and A. Ca, had renal arteriosclerosis. The narrowing of the lumina of the vessels in each patient was believed to have been sufficient to have reduced the blood flow through the kidneys. Evidence of interference with the blood supply to the tubules through the afferent glomerular vessels was seen in the microscopic sections from A. Ca. In J. Sm and A. Cas the

large renal vessels showed similar changes. It seems reasonable, therefore, to attribute a portion of the reduction in kidney function in these patients to reduced blood flow (31-32). In sixteen out of eighteen patients who died with gout Brogsitter (27) observed a systolic blood pressure greater than 170 and a diastolic greater than 104. Since his observations were collected late in the course of the disease we conclude that if hypertension occurs in gout it is a late manifestation secondary to prolonged renal damage and diminished renal flow.

The deposition of urates in the kidney parenchyma may be significant in the production of renal deterioration. In each of the kidneys which was studied at necropsy urates were visible grossly and microscopically. The medullary portions showed the most extensive deposits. In J. Sm. and A. Ca. the collecting tubules showed many small urate calculi. Most of these would be passed without producing symptoms of urinary tract obstruction, it might be the fate of others to develop into larger urate calculi. Obstruction and infection are possible sequelae.

The term nephritis has been avoided purposely in this discussion. The pathological diagnosis in the three patients studied at necropsy was chronic vascular nephritis. One should not quarrel about an anatomical diagnosis. The use of the term gouty nephritis in describing renal insufficiency in patients with gout is more hazardous. Many retain the term for late manifestations. Our data show that most patients with gout have some impairment of renal function. It is likely that the impairment is irreparable, although the progression appears to be very slow. A better term than gouty nephritis would be renal impairment of gout, which carries with it no special etiologic implications.

The term *urate clearance* has been employed rather freely in this discussion. It implies and embodies a process similar to the clearance of other substances which are excreted by the kidneys. Inherent aspects of urate clearance include appearance of urates in glomerular filtrate in approximately the same concentration as they exist in plasma, and absorption in part by contiguous cells as the glomerular filtrate passes through the tubular lumina. This theory assumes that excretion of urates by the tubules does not occur. The

elimination of urates by the kidney is of more than academic interest and once correctly defined possesses considerable etiologic significance. Future thinking and investigation concerning the etiology of gout will not be definitive until it is settled as to whether or not the kidneys are responsible for the accumulation of urates in patients with this malady. It was hoped that this study would help in the solution of the problem. That it has done but it has not clinched the argument. It is believed that our data show no differential inability of the kidneys to clear urate in gouty patients. This conclusion however, is based upon certain assumptions the proof for which is not yet available.

The urate clearance data of Berglund and Frisk (33) and Brøchner-Mortensen (34) were reported relative to creatinine clearance and not relative to inulin. If inulin is used as the standard a normal urate clearance is approximately 10 per cent of glomerular filtration. In gouty patients without impairment of renal function the urate clearance is similar. In gouty patients with a significant diminution in glomerular filtration, as measured by inulin clearance a quantitative decrease occurs in the reabsorption of urate by the tubules. The amount of urate excreted daily therefore shows little or no diminution.

There are at least three interpretations of the pathogenesis of depressed reabsorption. The cells of the tubules may be damaged and their efficiency lowered. An all or none law for the functioning of a nephron has not been demonstrated and it is likely that activity of a glomerulus and its tubule may be impaired without being completely destroyed. If the pathological processes causing kidney changes were to progress reabsorption in individual nephrons would approach zero. Patient H. Mc. (Table III) with advanced Bright's disease illustrates this. The inulin clearance was reduced to 85 cc. per minute while the urate was 7.5 cc. per minute. Reabsorption of urate from the glomerular filtrate was about 10 per cent instead of the normal of 90 per cent.

The second interpretation utilizes the hypothesis advanced by Hayman *et al.* (13). They have suggested that the failure of reabsorption of urea in dogs with damaged kidneys may be attributed to increased urine flow through the

possible that retarded reabsorption of urate in patients with impaired kidneys may be explained similarly. The abnormal data may represent the average of the combined filtration and reabsorption of normal and partially damaged nephrons.

In discussing this subject with Dr S J Thannhauser, he has called our attention to yet a third interpretation. If urates were excreted in part by the tubules, similar data might be obtained. This assumption is plausible if the premise were correct, that urates are excreted by the tubules. We believe that direct evidence in mammals and indirect evidence in man does not support this premise and that the explanation, therefore, must be held in abeyance for the present.

The action of drugs on renal function concludes the discussion. Cinchophen and salyrgan depress tubular reabsorption of urate, with subsequent increase in urate clearance. These changes may be demonstrated in patients with and without gout. They occur without significant change in inulin excretion. Patients with advanced renal insufficiency, however, do not show decreased urate reabsorption after cinchophen ingestion, as do those patients with little or no damage. It is believed that cinchophen damages normally functioning cells of the tubules and prevents reabsorption of urate just as salyrgan damages tubular cells and prevents reabsorption of urate as well as of sodium and chloride. Colchicine appears to have no effect on the renal excretion of urate. The pharmacological action of this drug in the treatment of acute or chronic gout is unknown.

SUMMARY

Kidney function has been investigated in twenty-two patients with gout. The function tests included accepted clinical procedures as well as clearance of inulin, creatinine, urate, sodium and chloride. Eight subjects with normal kidneys and one subject with terminal Bright's disease were used as controls. Most of the gouty patients showed some evidence of renal damage. The earliest change was inability to concentrate solids. In the absence of severe renal impairment, all except 10 per cent of the urates which were filtered through the glomeruli were reabsorbed by the tubules. With severe renal impairment, reabsorption of urates by the tubules was depressed and clearance tended to be maintained. No constitutional inferiority

of the kidneys to excrete urate was demonstrated. Cinchophen and salyrgan caused a diminution in tubular reabsorption of urate and an increase in urate clearance. Colchicine did not appear to influence the renal elimination of urates. *Kidney changes in patients with gout are believed to be the result and not the cause of the metabolic dyscrasia.*

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THE RELATION OF SPECIFIC GRAVITY TO COMPOSITION AND TOTAL SOLIDS IN NORMAL HUMAN URINE¹

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A study of the specific gravity of urine can make no pretense to novelty. The superiority of specific gravity determinations to uroscopy was pointed out by Cardinal Nicolaus of Cusa (Cusanus) in 1440. Leonhard Thurneysser of Thuring physician to Johann Georg von Brandenburg estimated the specific gravity of urine with a crude pycnometer (1576) and Methe (1727) devised a urinometer much like those in use today (13). The systematic study of the density of urine in kidney disease was described by Blackall in 1820 (6) who suggested that a low specific gravity might be due to a deficiency of urea in the urine. Bright and his associates (9) as well as Christison (10) Rayer (38) and Rees (39) gave increasing emphasis to its importance as an indication of the loss of concentrating power of the kidney. Hedinger and Schlayer (23) attempted to standardize conditions in order to obtain a urine of maximum concentration. Mosenthal (33) Volhard (42) Addis and Shevky (2) Lashmet and Newburgh (28) and Fishberg (14) introduced various modifications designed to simplify and increase the reliability of the concentration test. Fishberg especially has emphasized its clinical value designating it as "the most generally useful test of renal function for the general practitioner."

Many attempts have been made to estimate total solids of urine from specific gravity. All such efforts to discover an exact simple relationship presuppose either that each of the solids has the same effect per unit concentration or that the composition of the urine is always the same. Long (29) and Albarran (3) however pointed out the markedly different effects on specific gravity of equal concentrations (by weight) of the principal constituents of urine. Blohm (7) and Addis and Foster (1) concluded that no precise quantitative significance could be attached to the results of any method of estimating total solids of the urine from

specific gravity measurements because of its variable composition.

Pepper (35) found that, in normal subjects urea and chloride accounted for 50 to 75 per cent of the specific gravity of the urine but that the ratio of urea to chloride might vary considerably. Similar studies by Ishizu (24) on Japanese substantiated these findings. Alving and Van Slyke (4), on the basis of theoretical calculations without actual determination of the individual constituents in a given urine postulated that three-fourths of the rise in specific gravity above that of water may ordinarily be attributed to the mineral salts, and about one-fourth to urea with other organic solids of relatively slight influence. This statement was made in regard to urines of high gravity. Urines of low gravity were explained as being attributable in some cases to polyuria in others to a low output of salts. This emphasis on the gravity contribution of salts led these authors to conclude that the concentration test appears to measure chiefly the ability of the kidney to concentrate mineral salts.

Weiser and Thelen (43) on the other hand believed that the constituents of the urine could not alone account for the specific gravity and that volume effects or other physico-chemical phenomena would have to be invoked in order to account for the "residual specific gravity" (gravity unaccounted for by known constituents). Their work raises an important question: Is it necessary to postulate physico-chemical or other effects to explain the total urinary specific gravity or is the gravity of any urine a simple additive function of the concentrations of its constituents? If the latter is correct, then it should be possible to calculate the specific gravity of a urine from its composition.

This study concerns the relationship of the specific gravity to the composition of urine in normal subjects. It attempts to provide an answer to the following questions: Can the sp

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a complex dilute solution of inorganic and organic substances be calculated with accuracy? Can the specific gravity of a normal urine be similarly calculated from analysis of its composition? Is specific gravity a satisfactory index of total solids?

Specific gravity of complex aqueous solutions as an additive property of the contributions of the individual components

When a solid is dissolved in water, a slight increase in volume occurs, but the weight of water displaced is much less than the weight of the solute entering into the homogeneous liquid phase, so that the resulting solution has a greater weight per unit volume than the water alone. The mass per unit volume either of the solvent or of the solution is defined as the absolute density. Generally, however, the term "density" refers to the *relative* density, *i e*, to the ratio of the density of the solution to the density of a standard (usually water) either at the same temperature ($D_{\frac{t^{\circ}}{4^{\circ}}}$) or, more

frequently, at 4° ($D_{\frac{t^{\circ}}{4^{\circ}}}$). In either case, the value obtained is commonly called the specific gravity. $D_{\frac{t^{\circ}}{4^{\circ}}}$ is the preferable value since it is numerically equal to the density at t° , *i e*, it is the weight in grams per cubic centimeter at t° centigrade.

Since the terms "density" and "specific grav-

ity" are frequently rather loosely used, it should be emphasized that determinations of specific gravity are of no value unless the conditions under which they were obtained are recorded.

In the following discussion, the portion of specific gravity of a solution above the density of water similarly determined has been defined as the *specific gravity increment*. For water, $D_{\frac{t^{\circ}}{4^{\circ}}}$ is unity

and $D_{\frac{t^{\circ}}{4^{\circ}}}$ is the density at t° . Thus for an aqueous solution, $D_{\frac{t^{\circ}}{4^{\circ}}} - 1$ or $D_{\frac{t^{\circ}}{4^{\circ}}} - d_t$ (where d_t is the density of water at t°) gives the specific gravity increment. To be strictly comparable, since $D_{\frac{t^{\circ}}{4^{\circ}}} = D_{\frac{t^{\circ}}{4^{\circ}}} \times d_t$, the specific gravity increment of a solution of gravity $D_{\frac{t^{\circ}}{4^{\circ}}}$ should be given by $\frac{D_{\frac{t^{\circ}}{4^{\circ}}}}{d_t} - 1$. Rearranging this expression to $\frac{D_{\frac{t^{\circ}}{4^{\circ}}} - d_t}{d_t}$

shows that, since the numerator is small and the denominator approximately unity, the absolute error introduced by using the simpler expression is slight. In this study, $D_{\frac{20^{\circ}}{4^{\circ}}}$ is determined to the fourth decimal place and the above simplification affects the fifth decimal by less than five units, so the expression $D_{\frac{20^{\circ}}{4^{\circ}}} - 0.9982$ has been used to determine the specific gravity increments.

Before studying the relationship between specific gravity and concentration of solutes in aqueous solutions containing more than one dissolved substance, the effects of the individual solutes were investigated.

From the density tables of Landolt-Bornstein's Tabellen and the International Critical Tables the specific gravity increments of solutions containing 100 milli-equivalents (or milli mols) per liter at 20° were calculated, expressing the specific gravity as $D_{\frac{20^{\circ}}{4^{\circ}}}$. When the necessary data were not

available or recorded values did not agree, the increments were determined experimentally, using pycnometers of approximately 10 cc capacity. Since the error in the determination of specific gravity is less than 0.0001, the factors may be in error in the fifth decimal place but probably are all

TABLE I
Specific gravity factors

Values to be added to the specific gravity of water at $\frac{20^{\circ}}{4^{\circ}}$ (0.99823) to give the specific gravity at $\frac{20^{\circ}}{4^{\circ}}$ of a 100 milli-equivalent (or milli-molar) solution of the principal solutes found in urine.

	Increment per 100 mEq per liter						Increment per 100 mM per liter	
	Cl	SO ₄	H ₂ PO ₄	HPO ₄	HCO ₃	C ₁₂ H ₂ O ₇	Crea inine	Urea
Na	0.00413	0.00442	0.00591*	0.00675*	0.00610	0.00117		
K	0.00478	0.00700	0.00905*	0.00714*	0.00608			
NH ₄	0.00100	0.00388	0.00751	0.00349				
Ca	0.00484	0.00608	0.00860					
Mg	0.00385	0.00603						
							0.00785*	0.00102

* Determined experimentally. Other values calculated from density data of Landolt-Bornstein and International Critical Tables.

accurate to the fourth place. They are recorded in Table I

Since specific gravity increment varies directly with concentration in the lower concentration ranges, the values in Table I may be used to calculate the specific gravity of a solution of any of the above substances in the concentration range found in urine (from 20 to 300 mEq per liter for the inorganic constituents and from 100 to 600 mEq per liter for urea). Thus specific gravity $\frac{20^\circ}{4^\circ}$ of

1 per cent NaCl (171.2 mEq per liter) = 0.99823 + (1.712 × 0.00413) = 1.00529. The fifth decimal figure is significant only in placing the fourth figure to the closest unit.

Albarran's data (3) were derived in a like manner but calculated as grams of substance necessary to elevate the specific gravity 0.001 at 15°C when added to a liter of urine. Others (Addis and Foster (1), Pepper (35), Alving and Van Slyke (4), Ishizu (24), Willis (44), Weiser and Thelen (43), etc.) have made similar calculations.

In applying these factors to the calculation of the specific gravity of a strong solution or of a solution containing more than one solute, the total concentration of solute becomes the limiting factor in the accuracy of the calculation. Thus, each factor in the above table is calculated to allow for the displacement of a certain volume of water by the molecules of the solute when the latter is present in relatively low concentrations (1 to 2 per cent). If this concentration is sufficiently increased or if other solutes are added the volume of water in one liter of solution becomes progressively less and errors of increasing magnitude appear. In the case of NaCl the calculated specific gravity is in error by about 0.0004 at 4 per cent and 0.0010 at 6 per cent concentration. These values, however, are well above the concentrations of NaCl found in urine. The calculated specific gravity of a 6 per cent urea solution is in error by about 0.0001. From a similar consideration of the other factors above it may be estimated that the error in calculating the specific gravity contribution of a single substance in urine is 0.0001 or less. The use of factors based on molar rather than molal concentration eliminates the necessity of determining water content in each analysis.

Two implications are involved in applying sum

TABLE II
Calculated and observed specific gravities at $\frac{20^\circ}{4^\circ}$ of an artificial urine

Constituent	Concentration		Specific gravity increment
	grams per liter	mEq (mM) per liter	
Urea	27.923	465.0	0.00753
Creatinine	1.753	15.5	0.00044
KCl	3.989	53.5	0.00254
NaCl	4.530	77.5	0.00320
KH ₂ PO ₄	4.627	34.0	0.00328
Na ₂ HPO ₄	0.512	7.2	0.00049
K ₂ SO ₄	2.030	23.3	0.00163
(NH ₄) ₂ SO ₄	2.273	34.4	0.00133
Na C ₂ H ₃ O ₂	3.805	46.4*	0.00194
HC ₂ H ₃ O ₂	0.144	2.4*	0.00002
Water			0.99823
Specific gravity (calculated)			1.02063
Specific gravity (observed)			1.0202

* Acetate added as 48.8 cc. of N acetic acid + 46.4 cc. of N sodium hydroxide to obtain the ratio of salt to acid existing at pH 5.9

lar calculations to more than one solute. First, each individual substance must contribute its effect without influencing or being influenced by other substances, and second interchange of anions and cations must not affect the above factors.

A study of the latter assumption leads to an interesting observation pointed out by Valson (41). A mixture of 100 mEq of NaCl and 100 mEq of K₂SO₄ in one liter of solution has a calculated specific gravity $\frac{20^\circ}{4^\circ} = 0.99823$ (H₂O) + 0.00413 (NaCl) + 0.00700 (K₂SO₄) = 1.00936. Such a solution may equally well be considered as containing 100 mEq each of KCl and Na₂SO₄.

In this case, specific gravity $\frac{20^\circ}{4^\circ} = 0.99823$ (H₂O) + 0.00475 (KCl) + 0.00642 (Na₂SO₄) = 1.00940. The determined value is 1.0094. In other words the factor determined for each of the electrolytes in the above table is in reality a summation of two ionic factors each independent of the other. Further study of Table I reveals that this is apparently a general phenomenon. The five cations listed show differences in factors between Cl and SO₄ of 0.00229, 0.00225, 0.00222, 0.00214 and 0.00218, i.e. for any given cation as sulphate, a 100 mEq solution has a specific gravity at $\frac{20^\circ}{4^\circ}$

TABLE III

Calculated and observed specific gravity of urine after addition of salts and urea

Substance added	Concentration	Specific gravity increment of added substances	Specific gravity		Difference (Calculated—observed)
			Calculated	Observed	
	mEq (mM) per liter				
(Original specific gravity of urine = 1.0277)					
Urea	417.4	0.00676	1.0315	1.0338	0.0007
NaCl	205.4	0.00818	1.0362	1.0356	0.0005
K ₂ SO ₄	103.9	0.00727	1.0350	1.0317	0.0003
KH ₂ PO ₄	102.9	0.00993	1.0376	1.0372	0.0004
(Original specific gravity of urine = 1.0185)					
Urea	416.4	0.00674	1.0252	1.0216	0.0006
NaCl	205.5	0.00818	1.0270	1.0265	0.0005
K ₂ SO ₄	103.8	0.00727	1.0258	1.0254	0.0004
KH ₂ PO ₄	103.2	0.00996	1.0285	1.0279	0.0006

about 0.0022 higher than the same concentration of cation as chloride. The ionic nature of these specific gravity effects is not quite so exactly apparent throughout the table, particularly with some of the phosphates. This probably indicates partial ionization of the secondary and tertiary hydrogen. The cases where the agreement is poorest are those involving substances present in relatively small amounts in urine, so no attempt has been made to check these factors more closely.

In order to test the accuracy of specific gravity calculations of solutions approximating urine in composition, a number of "artificial urines" were studied. These were prepared by weighing the pure substances and dissolving them in water to a volume of one liter at 20°. Table II shows the composition and the calculated and observed specific gravities of one of these. This was a duplication of a normal urine, the composition of which had been determined by analysis, but the "artificial urine" contained only those substances for which specific gravity factors were available. Sodium acetate was used as representative of organic acids. The pH of this solution was 5.90, of the original urine, 5.88.

Five such "artificial urines" were studied with total solid contents ranging from 38.4 to 53.4 grams per liter. The maximum difference between calculated and observed specific gravity was 0.0007, the average 0.0004.

Another method of studying the applicability

of the above specific gravity factors to solutions of mixed solutes is to dissolve a substance in urine of previously determined specific gravity. Data from an experiment of this type are given in Table III. A weighed amount of solid was dissolved in urine, made up to a volume of 100 cc, and the specific gravity was determined by pycnometer and compared with the calculated value.

The average difference of 0.0005 between calculated and observed specific gravity is an indication of the approximate error involved in the calculation, and compares with the value 0.0004 observed as the average in "artificial urines." It represents the summation of the specific volume effects of the solutes. The first part of Table III represents specific gravities about as high as will be encountered in normal human urine, while the values in the second part are in the upper range of normal.

From this it may be concluded that the maximum error in calculating the specific gravity of a solution similar in composition to urine is less than 0.0010, and more likely of the order of 0.0005.

If Weiser and Thelen's postulate (43) that a significant portion of the specific gravity of urine is the result of volume contraction or other physicochemical effects be correct, these should diminish as the urine becomes more dilute. A concentrated urine in which such effects would be pronounced should on increasing dilution approach a straight-line relationship between total concentration and specific gravity increment in the range of low specific gravities although deviating significantly from linearity at higher specific gravities. This was not found to be the case. When a concentrated urine was diluted with water specific gravity increment was always a linear function of the degree of dilution, at least within two units in the fourth decimal place.

Calculation of the specific gravity of normal urine

Since the specific gravity of a complex solution with a total concentration of solutes of the order of magnitude found in urine can be calculated satisfactorily when the composition of the solution is known, an attempt can justifiably be made to calculate the specific gravity of urine from analysis of its major constituents.

Urine was collected from 6 normal males between the ages of 14 and 32. All subjects were given a weighed diet for a week before collections began and remained on that diet throughout the collection period. Two diets were used "high" protein containing 100 grams or 110

grams, and "low protein containing 40 grams or 50 grams daily. The remainder of the diet was adjusted to supply a total energy value of 2,300 to 3,000 calories. The daily ingestion of salt was kept approximately constant and the water intake varied to yield urines of specific gravity from 1.003 to 1.028.

Urine was collected over 24-hour periods in chemically clean glass jars containing toluene and stored during the collection period in a refrigerator at 3–6°C. The analyses were performed immediately following the close of the period.

Urine volume was measured in graduated cylinders of appropriate size, involving an error of less than 1 per cent. Specific gravities were determined with a Westphal balance at 20°C., using a bob calibrated to give D_{20}^{20} . By checking the balance from time to time, the maximum error in determination was kept below 0.0002. Protein as determined by the Shervy Stafford method (37) was not present in sufficient quantity to affect the observed specific gravity.

Hydrogen ion concentrations were determined in the earlier experiments by the bicolor method of Hastings, Sendroy and Robson (37). Later measurements were made with the Leeds and Northrup glass electrode assembly Number 7661-A1. When values in the upper pH range were anticipated, the urine specimens were collected with precaution against loss of CO₂ and stored under oil.

The analytical methods employed were total nitrogen by micro Kjeldahl urea, Van Slyke and Cullen or Van Slyke ammonia, aeration and titration creatinine, Folin colorimetric uric acid, Benedict and Franke potassium, Kramer and Tisdall sodium, Butler and Tutthill chloride, Volhard Harvey or Van Slyke and Sendroy sulphur (inorganic, etheral, and neutral) gravimetric methods of Folin and Benedict inorganic phosphate, Pincus and Malot organic acids Van Slyke and Palmer carbon dioxide content, Van Slyke (37) total base, Wright and Allison (45). The sum of calcium and magnesium was calculated by subtracting Na + K from total fixed base. The ratio of HPO₄ to H₂PO₄ was calculated from the pH. Bicarbonate was calculated from the total CO₂ by the Henderson Hasselbalch equation using the determined pH and the pK_a calculated from total base content according to Sendroy Seelig and Van Slyke (40a).

Total urine solids were determined by an application of the "cryochem" process of Flosdorf and Mudd (15). Ten cc. specimens of urine in glass evaporating dishes were frozen in an alcohol-solid CO₂ mixture and dehydrated for 24 hours in the frozen state in a vacuum desiccator over freshly dried granular calcium sulphate, using a Cenco "Hyvac" pump. With control solutions 24-hour drying was found to be sufficient to remove the water completely. Duplicate urine samples always checked at the end of this period but, with continued exposure to the high vacuum, the urine residues showed a progressive slow loss of weight amounting to about 1 per cent every 24 hours up to 96 hours.

This method of drying while largely obviating errors due to decomposition of the constituents of urine at higher

temperatures, results in the loss of volatile substances such as carbon dioxide from bicarbonate and volatile fatty acids. The continued removal of such substances is probably largely responsible for the decrease in weight after the first 24 hours.

The acid base balance of the electrolytes and the nitrogen partition were calculated as checks on the accuracy of the determinations.

From the urine analyses and the specific gravity factors (Table I) the increments of specific gravity contributed by each of the analyzed substances can be calculated and the sum compared with the observed total increment.

Three methods are available in handling the salts

(A) The various anions and cations may be combined in any convenient way to form salts, and the salt concentrations used in the calculation. As previously shown the method of combination is immaterial.

(B) The Na, K⁺ and NH₄⁺ ions may be partitioned among each of the anions according to their percentages of the total cation. For this purpose Ca⁺⁺ + Mg⁺⁺ are grouped with Na⁺. This grouping of Ca⁺⁺ + Mg⁺⁺ with Na⁺ is justified, as can be seen from the specific gravity factors of Table I. Ca⁺⁺ has a slightly greater and Mg⁺⁺ a slightly smaller effect on specific gravity than Na and since they are present in roughly the same concentration both being small the error in grouping them with Na is slight.²

(C) The anions may be considered as present entirely in the form of Na salts.

Methods A and B are both somewhat tedious but yield the same result. Method C which at first glance seems to be only a rough approximation, also gives the same value as A or B. This is due to a fortuitous balancing of the K and NH₄⁺ concentrations and factors and presumably would not always hold. If K or NH₄⁺ concentrations de-

² In a urine containing 30 mEq per liter of Ca + Mg (the maximum in our series) the error in calculating specific gravity by considering this fraction of cation as Na would be - 0.0012 if it were all Ca and + 0.00008 if it were all Mg. If present in the usual proportions the errors are almost completely balanced. This would not be true in urines where the cation distribution varies greatly from the normal (low salt intake, nephrosis, etc.) In such cases Ca and Mg should be determined as well as Na and K.

viate much from the normal, Method *A* or *B* is to be preferred

Table IV illustrates the calculation of the specific gravity of a mixed salt solution made up in approximately the same concentration as one of the normal urines. It will be seen that the three methods of calculation agree quite closely and are well within the limits of analytical errors.

The specific gravity of twenty normal urines from subjects on the standard diets was calculated by both Methods *B* and *C*, using the experimentally determined concentrations of Na, K, and NH_4 for *B*. Method *B* tends to give slightly lower values, but in no case does the difference exceed—0.0004. Fourteen of the twenty are identical or differ by 0.0001, seventeen do not differ by more than 0.0002.

Where Na and K were determined, Method *B* was used throughout. In some of the earlier experiments where these were not determined, Method *C* was used.

The experimental data from the first series of experiments on 2 male subjects with normal renal

function (ages 32 and 28) are given in Table V. The subjects were ambulatory during the experimental periods but exercise was limited in order to minimize salt and N loss by perspiration.

Since the acid-base balance involved eight analyses, a maximum difference between acidic and basic radicals of 10 per cent was arbitrarily chosen for the selection of urines for calculation of specific gravity. In most instances, the error was less than 5 per cent. On high protein diets, the sum of the cations is usually less than the sum of the anions, probably because the organic acids have been considered to be present entirely as salts (*i.e.*, as anions). On high protein diets, pH is consistently lower due to increased excretion of the acid products of protein catabolism. In these more acid urines, some of the organic acids may exist uncombined, amounting possibly to as much as 10 per cent at pH 5.0 (assuming an average pK of 4.0) and this nonionized fraction should not be used in calculating the acid-base balance. Since this correction cannot be accurately estimated, the organic acids have been grouped entirely with the anions,

TABLE IV
Comparison of methods for the calculation of specific gravity

Method A			Method B			Method C		
Constituent	Concentration	Specific gravity increment	Constituent	Concentration	Specific gravity increment	Constituent	Concentration	Specific gravity increment
KCl	mEq per liter 53.5	0.00254	KCl	mEq per liter 63.2	0.00300			
NaCl	77.5	0.00320	NH_4Cl	19.6	0.00033			
			NaCl	48.2	0.00199	NaCl	131.0	0.00541
K_2SO_4	23.3	0.00163	K_2SO_4	27.8	0.00195			
$(\text{NH}_4)_2\text{SO}_4$	34.4	0.00133	$(\text{NH}_4)_2\text{SO}_4$	8.7	0.00034			
			Na_2SO_4	21.2	0.00136	Na_2SO_4	57.7	0.00370
KH_2PO_4	34.0	0.00328	KH_2PO_4	16.4	0.00158			
			$\text{NH}_4\text{H}_2\text{PO}_4$	5.1	0.00038			
			NaH_2PO_4	12.5	0.00112	NaH_2PO_4	34.0	0.00304
Na_2HPO_4	7.2	0.00049	K_2HPO_4	3.5	0.00025			
			$(\text{NH}_4)_2\text{HPO}_4$	1.1	0.00004			
			Na_2HPO_4	2.6	0.00018	Na_2HPO_4	7.2	0.00049
H_2O		0.99823	H_2O		0.99823	H_2O		0.99823
Specific gravity		1.01070	Specific gravity		1.01075	Specific gravity		1.01087

Observed specific gravity = 1.0108

Method A Calculated according to composition as actually prepared

Method B Cations distributed proportionally among anions (Na = 36.8 per cent, K = 48.2 per cent, NH_4 = 15.0 per cent of total cation)

Method C Anions considered as being present entirely as sodium salts

TABLE V

Concentrations of major constituents and acid-base balance of twenty-two urines from 2 subjects on low and high protein diets

Subject	Volume	Specific gravity	pH	Urea	Creatinine	Total base	NH ₄	Cl	SO ₄	H ₂ PO ₄	HPO ₄	HCO ₃	Organic acid	Z Base	Z Acid	Base-acid	Error
	cc.			mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	mEq per liter	per cent
D (40 Gram protein diet) Age 30 Ht 173.5 cm Wt. 56.6 kgm																	
a	1500	1.0080	6.2	98.3	7.4	110.1	10.5	61.6	13.8	11.6	5.3	1.4	23.9	120.6	117.6	3.0	2.5
b	415	1.0283	6.3	296.0	23.7	349.5	26.6	167.9	54.1	40.2	23.2	2.0	107.2	376.1	394.6	-18.5	-4.8
c	460	1.0275	6.7	322.0	21.3	328.3	24.4	152.5	45.0	29.3	45.2	5.0	74.1	352.7	351.1	1.6	0.5
d	725	1.0162	6.4	199.8	15.4	216.4	21.5	111.5	28.1	20.4	14.9	0.9	40.8	237.9	216.6	21.3	9.4
e	3240	1.0030	6.6	51.9	3.2	58.7	5.5	32.5	6.1	4.5	5.4	3.1	9.2	64.2	60.8	3.4	5.5
f	2790	1.0037	6.5	52.2	3.8	68.0	6.1	36.6	7.6	6.8	6.3	2.8	10.2	74.1	70.3	3.8	5.3
D (100 Gram protein diet)																	
a	1000	1.0238	5.2	396.0	14.1	274.7	29.1	171.3	54.3	34.8	1.8	0.1	40.3	303.8	302.6	1.2	0.4
b	1020	1.0248	5.2	443.0	12.4	290.5	28.6	208.3	53.2	36.0	2.0	0.1	40.8	319.1	304.4	14.7	4.7
c	985	1.0275	5.1	472.0	11.7	284.5	30.6	189.8	60.0	38.6	1.8	0.1	32.8	315.1	323.1	-8.0	-2.5
d	1000	1.0266	5.2	503.0	13.4	314.9	29.6	215.3	59.8	33.7	1.8	0.1	40.0	344.5	350.7	-6.2	-1.8
e	3020	1.0064	5.0	160.0	4.2	80.4	16.0	63.3	18.7	10.5	0.4	—	11.1	96.4	104.0	-7.6	-7.6
f	3480	1.0063	5.2	146.0	3.5	92.1	11.0	66.0	17.5	9.7	0.3	—	11.1	103.1	104.9	-1.8	-1.7
g	1640	1.0136	5.2	311.0	8.6	153.8	20.1	90.0	34.7	22.5	1.2	0.1	21.8	173.9	170.3	3.6	2.1
h	1790	1.0127	5.2	283.0	7.8	154.6	19.6	104.7	33.3	20.2	1.0	0.1	26.5	174.2	185.8	-11.6	-6.4
M (50 Gram protein diet) Age 28 Ht 180 cm Wt. 72.5 kgm																	
a	890	1.0217	6.6	300.6	18.6	255.8	35.1	148.8	39.1	20.6	24.8	3.5	43.1	290.9	279.9	11.0	3.9
b	580	1.0265	6.4	355.0	23.1	300.9	28.8	188.4	45.1	26.3	19.2	4.0	48.7	329.7	331.7	-2.0	-0.6
c	1980	1.0080	6.7	128.5	7.7	124.5	10.7	75.9	14.5	7.1	10.8	7.6	17.8	135.2	133.7	1.5	1.1
d	1410	1.0110	6.6	161.6	10.2	149.3	15.1	92.3	20.0	10.1	12.2	4.5	23.0	164.4	162.1	2.3	1.4
M (110 Gram protein diet)																	
a	1635	1.0171	5.7	311.7	9.3	195.7	26.4	140.2	38.3	23.4	3.2	0.9	23.9	222.1	229.9	-7.8	-3.5
b	1160	1.0229	5.7	396.0	14.8	265.1	32.8	205.9	50.4	27.7	3.8	0.6	33.1	297.9	321.5	-23.6	-7.6
c	2350	1.0105	6.2	225.0	6.8	136.0	14.1	86.3	26.0	12.7	5.8	2.0	16.8	150.1	149.6	0.5	0.3
d	2920	1.0080	5.7	182.5	5.6	91.1	13.6	55.1	21.8	13.2	1.8	0.5	14.4	104.7	106.8	-2.1	-2.0

giving, in the more acid urines a total concentration of acidic constituents bound to base slightly in excess of the amount actually present.

Using the factors given in Table I the specific gravity contributions of urea, creatinine, chloride sulfate, phosphate and bicarbonate were calculated by Method C. Data of the 2 subjects D and M are presented in Table VI. In addition to these 2 subjects, similar studies were carried out on 5 more individuals (subject M was studied again 6 months after the first experiments). The average per cent of specific gravity determined on the two protein diets is given in Table VII. (Method B was used in calculations in the last four subjects.)

In a person with normal kidney function on a relatively constant diet the specific gravity con-

tributions of the substances enumerated above are fairly constant since they are for the most part derived from exogenous sources and during a 24-hour period will be almost completely excreted, provided the subject is in balance. Urine volume changes over normal ranges will not affect output appreciably, consequently these substances will be diluted or concentrated to the same degree and the specific gravity effect expressed on a percentage basis will remain constant. Over a range of specific gravity from 1.0030 to 1.0283 on either low or high protein diet the same specific gravity pattern is obtained.

On a high protein diet relatively more of the specific gravity can be accounted for the total averaging between 80 and 90 per cent, as compared

TABLE VI

Calculations of specific gravity (method C) from data of Table V, using factors of Table I

Subject	Specific gravity increment	Contributions to specific gravity of														Sum of specific gravity contributions	Per cent specific gravity determined
		Urea	Per cent	Creatinine	Per cent	NaCl	Per cent	Na ₂ SO ₄	Per cent	NaH ₂ PO ₄	Per cent	Na ₂ HPO ₄	Per cent	NaHCO ₃	Per cent		
D (a)	0.0098	0.00159	16.2	0.00021	2.1	0.00254	25.9	0.00089	9.1	0.00104	10.6	0.00036	3.7	0.00009	0.9	0.00672	68.6
(b)	0.0301	480	16.0	68	2.3	694	23.1	347	11.5	359	11.9	157	5.2	12	0.4	0.02117	70.2
(c)	0.0293	522	17.8	61	2.1	630	21.5	289	9.9	262	8.9	305	10.4	30	1.2	0.02099	71.6
(d)	0.0180	324	18.0	44	2.4	461	25.6	180	10.0	182	10.1	100	5.6	5	0.3	0.01296	72.0
(e)	0.0048	84	17.5	09	1.9	135	28.1	39	8.1	40	8.3	36	7.5	19	4.0	0.00362	75.2
(f)	0.0055	85	15.5	11	2.0	151	27.5	49	8.9	61	11.1	43	7.8	17	3.1	0.00417	75.8
D (a)	0.0256	0.00642	25.1	0.00040	1.6	0.00708	27.7	0.00349	13.6	0.00311	12.1	0.00012	0.5	0.00001		0.02063	80.6
(b)	0.0266	718	27.0	35	1.3	861	32.4	342	12.9	322	12.1	13	0.5	1		0.02292	86.2
(c)	0.0293	764	26.1	33	1.1	784	26.8	385	13.1	345	11.8	12	0.4	1		0.02324	79.4
(d)	0.0284	815	28.7	38	1.3	890	31.3	384	13.5	301	10.6	12	0.4	1		0.02441	86.0
(e)	0.0082	259	31.6	12	1.5	261	31.8	120	14.6	94	11.5	3	0.4	0		0.00749	91.4
(f)	0.0081	237	29.3	10	1.2	273	33.7	112	13.8	87	10.7	2	0.2	0		0.00721	89.0
(g)	0.0154	504	32.7	25	1.6	372	24.2	223	14.5	201	13.1	8	0.5	1		0.01334	86.6
(h)	0.0145	459	31.7	22	1.5	433	29.9	214	14.8	181	12.5	7	0.5	1		0.01317	90.8
M (a)	0.0235	0.00487	20.7	0.00053	2.3	0.00614	26.3	0.00251	10.7	0.00184	7.8	0.00167	7.1	0.00021	0.9	0.01777	75.6
(b)	0.0283	575	20.3	66	2.3	778	27.5	290	10.2	235	8.3	130	4.6	24	0.8	0.02098	74.1
(c)	0.0098	208	21.2	22	2.2	314	32.0	93	9.5	63	6.4	73	7.5	46	4.7	0.00819	83.6
(d)	0.0128	262	20.5	29	2.3	381	29.8	228	10.0	90	7.0	82	6.4	27	2.1	0.00999	78.0
M (a)	0.0189	505	26.7	26	1.4	579	30.6	246	13.0	209	11.1	22	1.2	05	0.3	0.01592	84.2
(b)	0.0247	642	26.0	42	1.7	850	34.4	324	13.1	248	10.0	26	1.1	04	0.2	0.02136	86.5
(c)	0.0123	364	29.6	19	1.5	357	29.0	167	13.6	114	9.3	39	3.2	12	1.0	0.01072	87.2
(d)	0.0098	296	30.2	16	1.6	228	23.3	140	14.3	118	12.0	12	1.2	03	0.3	0.00813	83.0

with 70 to 80 per cent on a low protein diet (Table VII). An increase in protein intake results in a greater output of urea, sulfate and phosphate without a proportional increase in water, that is, the normal human kidney seems to have the ability to eliminate successive increments of these substances with some economy of water (*cf* Gamble (19)). Their specific gravity contributions necessarily become proportionately greater, except in the case of phosphate where the increased molar concentration may be offset by the decrease in equivalent concentration resulting from the shift from alkaline to acid salts at the lower pH values.

The chloride fraction of specific gravity represents $\frac{1}{3}$ to $\frac{1}{2}$ of the total. In general, the chloride contribution to specific gravity was slightly less with the high protein diet since the salt intake was only slightly increased.

Creatinine contributed only a small quantity (1 to 2 per cent), which was definitely less on the higher protein diet. Since creatinine is essentially a product of endogenous metabolism and since more solids are excreted on a high protein diet, creatinine makes up a smaller percentage of the total.

Bicarbonate was variable but usually did not comprise a very significant part of the total. The excretion of bicarbonate is dependent on pH and CO₂ tension and at the acidity of normal urine and under a normal tension will not generally exceed 10 mEq per liter (18, 40b).

These data indicate that 70 to 90 per cent of the specific gravity of the urine from normal subjects on varying protein diets can be accounted for by chloride, urea, sulfate, phosphate, creatinine, and bicarbonate. Of these, chloride contributes roughly 25 per cent, sulfate and phosphate together 20 to 30 per cent, urea 15 to 30 per cent, creatinine 1 to 2 per cent, and bicarbonate 0 to 5 per cent. Urea, sulfate, and phosphate, comprising the major derivatives of protein metabolism, make up a greater fraction on high protein diets.

The undetermined fraction of specific gravity

The remaining 10 to 30 per cent of the specific gravity unaccounted for on the basis of analyses is a fraction of much interest. In specific gravity units, it amounts to 0.0007 to 0.0061 on the high protein diet, and 0.0012 to 0.0089 on the low pro-

tein diet, the absolute magnitude depending on the total gravity. Since it has been shown that in a solution such as urine interionic or other physico-chemical factors do not significantly affect specific gravity this relatively large undetermined fraction must be due to constituents of urine not determined by analysis.

For a more detailed study of this undetermined fraction another series of nine normal urines from 4 subjects was analyzed. The conditions of diet collection of samples analytical methods etc. were the same as for the previously studied series. In addition Na, K, total solid, total carbon and ash were determined. The analytical data were treated by Method B (Table IV) for the calculation of specific gravity. The concentration of each substance analyzed was also expressed in grams from which the 24-hour output of determined substances could be calculated and the sum of these compared with the total solid output. The determined 24-hour output was further divided into organic (urea + creatinine) and inorganic fractions and these were compared with the

TABLE VIII
Method of calculating specific gravity and output of solids of urine

Subject: B (May 23, 1939)		24-Hour Vol. = 1280 cc.		$D_{20}^{20} = 1.0148$	
Total base.	100.0 mEq. per liter	Cl	108.0 mEq. per liter		
K.	64.1	SO ₄	34.9		
Na.	104.3	H ₂ PO ₄	7.6		
Ca+Mg.	21.6	HPO ₄	18.7		
Ammonia	18.8	HCO ₃	14.4		
	208.8 mEq. per liter	Organic acids	33.6		
		207.3 mEq. per liter			
Total solids = 34.1 grams per liter ash = 31.7 per cent.					
NH ₃ = 8.0 per cent, K = 30.7 per cent, Na+Ca+Mg = 60.3 per cent of total solids.					

Substances	Concentration	Specific gravity increment	Output
	mEq. per liter		grams per liter
KCl	33.3	0.00158	2.473
NH ₄ Cl	9.7	0.00016	0.319
NaCl	65.1	0.00269	3.805
K ₂ SO ₄	7.7	0.00054	0.471
(NH ₄) ₂ SO ₄	2.3	0.00009	0.145
Na ₂ SO ₄	15.0	0.00096	1.065
KH ₂ PO ₄	2.3	0.00023	0.319
(NH ₄)H ₂ PO ₄	0.7	0.00005	0.081
NaH ₂ PO ₄	4.6	0.00041	0.823
K ₂ HPO ₄	4.8	0.00084	0.418
(NH ₄)HPO ₄	1.4	0.00003	0.003
NaH ₂ PO ₄	8.5	0.00084	0.878
KHCO ₃	4.4	0.00029	0.440
(NH ₄)HCO ₃	1.3	0.00008	0.103
NaHCO ₃	8.7	0.00033	0.731

Sum of determined inorganic solids 12.056

Urea	mM. per liter	0.00353	13.463
Creatinine	11.7	0.00033	1.323

Sum of determined organic solids 14.786

Total.		0.01237	26.873
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Specific gravity determined = $\frac{0.01237}{1.0148 - 0.9982} \times 100 = 77.3$ per cent

Solids determined = $\frac{26.8}{34.1} \times 100 = 78.6$ per cent

24-Hour output:

1. Total solids 34.1 × 1.280 = 43.6 grams
2. Determined solids 26.8 × 1.280 = 34.3 grams
3. Undetermined solids 4.5 = 34.3 - 11.6 grams
4. Ash 0.317 × 44.8 = 14.4 grams
5. Determined inorganic solids 12.1 × 1.280 = 15.5 grams
6. Organic solids (Total solids - ash) 4.5 - 14.4 = -11.1 grams
7. Determined organic solids 14.5 × 1.280 = 18.6 grams

corresponding values calculated from the total solid and ash determinations. An example of the calculation is given in Table VIII and the data for all of the urines in this series are summarized in Table IX.

The data of Table IX show the following relationships between the determined fraction of specific gravity and the determined solid constituents of the urine.

(1) The per cent of specific gravity accounted for by the major constituents of urine is a $\frac{1}{2}$ the

TABLE VII

Average determined percentage of specific gravity of urines from subjects on varying protein diets

Subject	Number of observations	Nitrogen output	Per cent of specific gravity accounted for by						Total
			Urea	Cl	SO ₄	PO ₄	HCO ₃	Creatinine	
		grams							per cent

40-50 Grams protein diet

Lom.	6	4.6*	12.0	42.4	6.2	10.5	3.9	1.6	76.6
D	6	5.4*	16.8	25.3	9.6	16.9	1.7	2.1	72.4
M	4	8.4	20.7	28.9	10.1	13.8	1.6	2.3	77.4
M†	1	9.4	23.4	30.1	10.0	10.3	1.3	2.0	77.1
L	2	8.7	20.4	27.2	10.3	9.9	5.4	1.9	75.1
B	2	9.7	21.4	27.8	9.8	11.4	4.7	2.0	77.1
K	2	11.1*	24.4	22.8	11.9	13.0	1.0	2.2	75.3

100-110 Grams protein diet

Lom.	7	10.5*	19.6	36.6	9.9	11.5	1.9	1.2	80.7
D	8	15.4	29.0	29.7	13.9	12.2	0.0	1.4	86.2
M	4	16.6	28.1	29.3	13.5	12.3	0.5	1.6	85.3
M†	1	16.7	28.3	27.5	14.2	13.9	0.2	1.6	85.7
L	1	16.4	30.2	22.5	14.7	14.9	0.5	1.5	84.3
B	2	17.3	29.7	22.6	13.4	13.8	0.5	1.6	81.6
K	2	17.5	30.3	19.2	14.2	14.4	0.2	1.7	80.0

* Not in nitrogen balance

† Six months after first set of observations.

TABLE IX

Percentage of specific gravity and total organic and inorganic solids determined by analysis of nine normal urines

Subject	Date	24-Hour volume	Specific gravity increment observed	Specific gravity increment calculated	Per cent specific gravity determined	Total solids output	Determined solids output	Per cent determined	Ash per cent of total solids	Ash output	Determined inorganic solids output	Inorganic solids per cent of ash	Organic solids (total solids—ash) output	Determined organic solids output	Per cent organic solids determined
		cc				grams	grams			grams	grams		grams	grams	
"High" protein diet															
M	April 17	1770	0 0167	0 01436	86 0	68 1	55 8	82 0	31 8	21 7	23 0	106 0	46 4	32 8	70 7
L	April 18	1585	0 0176	0 01489	84 6	65 8	53 2	80 9	29 8	19 6	20 4	104 1	46 2	32 8	71 0
B	May 19	1520	0 0200	0 01614	80 7	72 4	57 0	78 7	27 2	19 7	20 5	104 1	52 7	36 4	69 1
K	May 23	1445	0 0200	0 01568	78 4	68 8	53 5	77 8	26 1	18 0	18 6	103 3	50 8	34 8	68 5
Average					82 4			79 9				104 4			69 8
"Low" protein diet															
L	April 24	1300	0 0155	0 01094	70 6	41 7	30 4	72 9	32 2	13 4	13 4	100 0	28 3	17 0	60 1
L	April 25	1430	0 0144	0 01151	79 9	43 7	33 2	76 0	35 4	15 5	16 4	105 8	28 2	16 7	59 2
M	April 28	1320	0 0157	0 01213	77 2	44 3	34 7	78 4	33 6	14 9	15 2	102 0	29 4	19 7	67 0
B	May 25	1260	0 0163	0 01260	77 3	45 5	33 9	74 5	31 7	14 4	15 2	105 6	31 1	18 6	59 8
K	June 1	960	0 0216	0 01645	76 2	47 7	35 1	73 6	30 7	14 6	14 3	98 0	33 1	20 9	63 2
Average					76 2			75 1				102 3			61 9

almost identical with the per cent of total solids which these constituents comprise. There is, therefore, present in urine a very appreciable quantity of solid matter, not ordinarily determined by analysis, but probably sufficient to account for the remainder of the specific gravity if its composition and the specific gravity contributions of the component substances were known.

(2) The determined inorganic solids include all compounds present in sufficiently high concentration to have a significant effect either on specific gravity or on total solid content. The calculated acid-base balances serve as a check on these analyses. As a further check, the 24-hour output of inorganic substances is found to be 98 to 106 per cent of the ash content of the urines, as determined by dry ashing of the residues obtained in the total solid determination. Since this method of ashing produces little change in the composition of these salts,³ this agreement indicates that the inorganic

constituents have been practically completely determined. The undetermined solid is, therefore, organic in nature.

(3) Urea and creatinine account for only 60 to 70 per cent of the total organic matter (total solids-ash). Approximately 30 to 40 per cent of the organic constituents are substances not commonly determined in urine analyses and they may quite readily account for the 10 to 30 per cent of undetermined specific gravity.

(4) The absolute amount of undetermined organic solids is 10 to 15 grams in the 24-hour urines, slightly higher with high protein intake (Table X).

Direct study of this 10 to 15 grams would necessitate the estimation of a great number of substances excreted in the urine in amounts varying from a few milligrams to a gram or more per day. Such analyses would be impractical and even impossible on a single 24-hour specimen, consequently only the general nature of the undetermined fraction will be discussed. Table X summarizes the data for this fraction from the series of urines presented in Table IX.

One large group of compounds, the organic change in weight during the ashing so that the ash content of a urine may be used as a fairly accurate index of the mineral salts present.

³ Dry ashing will not appreciably affect alkali or alkaline earth chlorides and sulfates. There will be some loss of water from phosphates with formation of metaphosphates and decomposition or volatilization of ammonium salts. Salts of organic acids (which have not been included in calculating the total output of inorganic solids) will be converted to carbonate or bicarbonate and the cations will largely recombine with the anions previously held by ammonia. The net result is a relatively slight

acids has been determined in all of the analyses and is recorded in Tables V and X. This analysis is essential in calculating the acid base balance but the contribution of organic acids to specific gravity has not been calculated since the individual organic acids are not sufficiently well known to make possible the use of an accurate specific gravity factor.

Of the acids determined collectively as organic acids, uric, hippuric and citric are known to occur in appreciable amounts. Of these, only uric acid has been determined, and its contribution to specific gravity cannot be estimated since its physical state in urine is not known it frequently being present in concentration greater than its true solubility in water either as free acid or as urate (36). Hippuric acid is found in appreciable amounts (0.8 to 1.9 grams daily output (17-25)) and citric acid may be excreted in amounts from 0.2 to 1.0 gram daily (8-34). However the excretion of these known acids can rarely exceed 3.0 grams per day, which is about 16 mEq or $\frac{1}{3}$ to $\frac{1}{2}$ of the total organic acid excretion determined by the method of Van Slyke and Palmer (37). If the unknown acids have about the same average equivalent weight as these three, the organic acid fraction could well amount to 6 to 10 grams per day or about half of the undetermined solids.

A very rough idea of the general effect of organic acids on specific gravity may be calculated from the density data of the International Critical Tables. As sodium salts these acids have specific gravity factors of about 0.004 to 0.009 per 100 mEq per liter averaging about 0.006. This would definitely place them as comparable to the inorganic salts in their effect on specific gravity (*cf* Table I). Assuming the urinary organic acids to behave in a similar manner the 10 mEq per liter to 100 mEq per liter present might total 0.0006 to 0.006 in specific gravity units which accounts for about $\frac{1}{2}$ of the undetermined fraction of specific gravity.

The fractions of sulphur designated as ethereal and neutral sulphur are listed in Table X. The former group contains sulphuric acid conjugated with phenolic compounds such as phenol, indoxyl, skatolyl, p-cresol and possibly others such as pyrocatechol and hydroquinone some of which may be weak enough acids to be at least partially determined in the organic acid fraction. The neut-

ral sulphur is present in a wide variety of compounds such as cystine, thiocyanates, taurine derivatives, oxypyroteic acids, etc. which are largely endogenous.

While some ethereal compounds of phosphoric acid are present in urine, the amount is so small as to comprise 5 per cent or less of the total phosphorus (30-46). This has been verified in some of the urines studied and the amount of phosphorus represented by these compounds has been neglected.

Small amounts of fermentable carbohydrate are present even in normal urine but the total reducing power of urine is probably largely due to non-carbohydrate substances. Pigments are constantly present, about 75 mgm being excreted daily (12). It is obviously impractical to assign specific gravity factors to these and the multitude of other substances which have been found in urine.

From the data of Table X, the 24-hour output of undetermined C, N and S can be compared with the output of undetermined solid. Uric acid was determined in all of these urines but was grouped with the organic acids which were not considered in calculating the specific gravity. Carbon makes up about 35 to 44 per cent of the weight, nitrogen 5 to 10 per cent, neutral sulphur 0.5 to 1.0 per cent and ethereal sulphur 0.3 to 7 per cent. This

TABLE X
Comparison of 24-hour output and composition of total and of undetermined solids

Subject	Date	Total solids			Undetermined solids*					
		Output	Carbon	Nitrogen	Output	Carbon	Nitrogen	Ethereal sulphur	Neutral sulphur	Organic acids
		grams	grams	grams	grams	grams	grams	grams	grams	mEq.
"High protein diet"										
M	April 17	68.1	11.30	16.06	12.3	4.30	0.97	0.034	0.065	47.2
L	April 18	85.8	11.19	16.44	12.6	4.23	0.86	0.066	0.087	40.0
B	May 19	72.4	12.43	18.42	18.4	8.71	1.04	0.035	0.078	53.5
K	May 23	68.8	12.73	17.78	12.3	8.23	1.15	0.073	0.064	56.0
Low protein diet										
L	April 24	41.7	8.07	8.58	11.3	4.18	0.63	0.043	0.023	34.4
L	April 25	42.7	8.04	8.62	10.5	4.09	0.64	0.041	0.026	35.9
M	April 28	44.3	8.11	8.57	9.8	4.14	0.53	0.035	0.024	34.9
B	May 28	45.5	8.78	9.20	11.9	4.48	1.03	0.067	0.025	46.1
K	June 1	47.7	8.33	10.84	12.6	4.73	1.02	0.061	0.074	41.3

* Ethereal sulphur, neutral sulphur and organic acids have been considered entirely with the undetermined fraction of total solids.

corresponds to an atomic ratio of one atom of sulphur for 10 to 18 atoms of nitrogen and 73 to 107 atoms of carbon⁴. Such a calculation is obviously of no value in determining the composition of any single constituent of the undetermined organic fraction nor is it useful in calculating a specific gravity factor for the unknown fraction. It does, however, permit the following comments

(1) The undetermined fraction as a whole contains a smaller percentage of nitrogen than if it were composed entirely of protein degradation products. If organic acids, which probably contain little or no nitrogen make up one-half or more of this fraction, the remainder might well approach the composition of protein fairly closely.

(2) Since sulphur makes up only about 1 to 1.5 per cent of the undetermined solid, and the known sulphur-containing compounds in the urine have a relatively high percentage of this element, the actual weight of these compounds must be small.

(3) The percentage composition of the undetermined fraction in the 4 subjects studied on both low and high protein diets is remarkably constant. The absolute quantity excreted daily is only slightly higher on the latter regime. This slight increase may easily be due to the greater excretion of organic acids. These facts indicate quite strongly that the undetermined substances are largely endogenous.

Dialysis of urine through cellophane membranes gives an indication of the molecular size of the substances present. When dialyzed against a confined volume of water, all of the substances analyzed are found to be freely diffusible. When dialyzed against running water until the dialysate is free of chloride, 100 cc of a urine originally containing 1.13 grams of nitrogen and 57 grams of total solid contained only 2.9 mgm of nitrogen and 38 mgm of solid. The specific gravity was the same as that of water within the limit of error.

⁴ If the uric acid is not included in the undetermined fraction, the undetermined solid is reduced by 0.5 to 0.8 gram but the percentage composition and empirical formula are not changed significantly (carbon 33 to 40 per cent, nitrogen 4 to 9 per cent, neutral sulphur 0.6 to 1.1 per cent and ethereal sulphur 0.3 to 0.8 per cent, corresponding to an atomic ratio of 1 sulphur to 7 to 14 nitrogen and 70 to 103 carbon).

TABLE VI

Calculation of specific gravity factor for undetermined fractions of organic solids (A), and comparison of these factors with those of known substances in urine (B)

(A)

Subject	Solids undetermined grams per liter	Specific gravity undetermined	Factor effect 1 gram per liter
110 Gram protein diet			
B	13.1	0.0040	0.00031
B	10.2	0.0039	0.00038
K	14.9	0.0048	0.00032
K	10.7	0.0044	0.00041
M	6.9	0.0024	0.00035
L	8.0	0.0028	0.00035
Average			0.00035
50 Gram protein diet			
B	9.2	0.0037	0.00040
B	8.9	0.0035	0.00039
K	13.1	0.0050	0.00038
K	13.0	0.0052	0.00040
M	7.2	0.0036	0.00050
L	8.7	0.0046	0.00053
L	7.3	0.0030	0.00041
Average			0.00043
Combined average			0.00039

(B)

Substance	Specific gravity factor increment per gram per liter	Substance	Specific gravity factor increment per gram per liter
NaCl	0.00071	NH ₄ Cl	0.00031
KCl	0.00064	(NH ₄) ₂ SO ₄	0.00059
Na ₂ SO ₄	0.00090	(NH ₄) ₂ H ₂ PO ₄	0.00065
K ₂ SO ₄	0.00080	(NH ₄) ₂ HPO ₄	0.00053
NaH ₂ PO ₄	0.00074		
KH ₂ PO ₄	0.00071		
Na ₂ HPO ₄	0.00095	Urea	0.00027
K ₂ HPO ₄	0.00082	Creatinine	0.00025

of determination. From these two experiments it is evident that normal urine contains only inappreciable quantities of true colloidal or non-dialyzable substances. Consequently, such compounds cannot contribute significantly to the undetermined specific gravity or to the total solids. What little is present is probably mucus or cellular elements derived from the urinary passages plus a small amount of pigment.

The undetermined substances of the urine may, therefore, be characterized as (1) organic compounds, (2) of relatively low molecular weight (dialyzable), (3) relatively low in nitrogen content (4) showing only slight variation with diet and (5) sufficient in quantity to account for the undetermined fraction of specific gravity

The average specific gravity effect per gram of undetermined solid can be obtained from the data presented. This calculation was carried out in thirteen instances and the results are tabulated in Table XI-A. In Table XI-B the increments per gram of the determined substances in urine are shown for comparison. The latter fall in three distinct groups: the inorganic salts of Na and K having the greatest effect per gram, urea and creatinine the smallest, and the ammonium inorganic salts intermediate. The undetermined solids exert an effect closest to the organic substances (urea and creatinine) which is consistent with their organic nature. On the high protein diets the undetermined solids have a slightly higher factor, but the difference is not significant. The constancy of this factor is in keeping with a belief in the relative constancy in composition of the undetermined solids.

Estimation of total solids of urine from specific gravity with a critical evaluation of Häser's and Long's coefficients

Since the specific gravity of urine is a simple additive function of the concentrations of the individual constituents there is theoretical justification for the various empirical coefficients proposed for the estimation of the solids of urine from specific gravity. Christison in 1840 (10) seems to have been the first to propose such a coefficient, but it was not until Häser's (21) work (1854) that the method received much attention. The latter estimated the total urinary solids in grams per liter by multiplying the second and third decimal figures of the specific gravity by 2.33. Long (29) determined specific gravity to the fourth place by pycnometer and the total solids by an evaporation method which permitted correction for losses due to the ammonia evolved from urine on heating. His coefficient of 0.260 at 25/4° is the one most

frequently used at the present time, and is quoted by Hawk and Berghem (1937) (22) and Best and Taylor (1939) (5). Long calculated his coefficient by dividing the weight of solids by the last three figures of the specific gravity. He noted, without explanation, that there is more variation in the coefficient at low gravities, a variation due in part to the fact that the solid content is correlated not with the last three figures of specific gravity but with the actual increment of specific gravity above that of water. With lower gravities this correction plays a significant role, as is shown in the following example. A urine of 1.0300 gravity using Long's coefficient of 0.260 should contain 78.00 grams of solid per liter. It has been shown experimentally in the early part of this paper that if such a urine is diluted with water the specific gravity increment is an inverse linear function of the degree of dilution. Since Long determined

$D_{4^{\circ}}^{25^{\circ}}$, the specific gravity increment in this case is $1.0300 - 0.9971 = 0.0329$. If this urine were diluted four fold with water at 25° the specific gravity $D_{4^{\circ}}^{25^{\circ}}$ would be $0.9971 + \frac{0.0329}{4} = 1.0053$

not $1.0000 - \frac{0.0300}{4} = 1.0075$, as is implied when concentration is expressed as a function of the decimal figures of the specific gravity. Since the total solid in the diluted urine is 19.5 grams per liter, Long's coefficient calculated for this example would be $\frac{19.5}{53} = 0.368$. Using the average coefficient of 0.260 for this urine, the estimated solids would be $0.260 \times 53 = 13.8$ grams per liter, almost 30 per cent low.

Long's coefficient was derived from normal urines the great majority of which were of high specific gravity (only four of the fifty-two urines studied had specific gravities below 1.015, ten were between 1.015 and 1.020, the rest above 1.020). With these urines of high gravity the absolute error is not so significant. With the urines of low gravity frequently obtained in disease, however, Long's coefficient will give values for total solid as much as several hundred per cent in error. This criticism does not apply to the coefficient determined from $D_{4^{\circ}}^{t^{\circ}}$ (7).

* The literature has been reviewed by Blohm (7)

TABLE XII

Calculation of coefficients for estimation of total solid content of urine from observed specific gravity (A), and comparison of these with similar coefficients of individual constituents of urine (B)

(A)

Subject	Total solid grams per liter	$\frac{I}{\text{Specific gravity increment}}$	Coefficient $\frac{S}{I \times 10,000}$
110 Gram protein diet			
B	55.4	0.0227	0.244
B	47.6	0.0200	0.238
K	62.3	0.0253	0.246
K	47.7	0.0200	0.239
M	38.5	0.0167	0.231
L	41.5	0.0176	0.236
Average			0.239
50 Gram protein diet			
B	36.1	0.0163	0.221
B	32.9	0.0150	0.219
K	49.5	0.0216	0.229
K	44.7	0.0194	0.231
M	33.5	0.0157	0.213
L	32.1	0.0155	0.207
L	30.5	0.0144	0.212
Average			0.219
Combined average			0.227

(B)

Substance	Coefficient $\frac{S}{I \times 10,000}$	Substance	Coefficient $\frac{S}{I \times 10,000}$
NaCl	0.142	NH ₄ Cl	0.322
KCl	0.157	(NH ₄) ₂ SO ₄	0.170
Na ₂ SO ₄	0.111	(NH ₄) ₂ H ₂ PO ₄	0.153
K ₂ SO ₄	0.125	(NH ₄) ₂ HPO ₄	0.189
NaH ₂ PO ₄	0.134		
KH ₂ PO ₄	0.141		
Na ₂ HPO ₄	0.105		
K ₂ HPO ₄	0.122		
NaHCO ₃	0.138	Urea	0.371
KHCO ₃	0.150	Creatinine	0.397

specific gravity increment is identical with the decimal figures of the observed specific gravity

Aside from this theoretical objection to Long's coefficient, there was no great constancy even among urines of high specific gravity, the value ranging from 0.234 to 0.288, a variation of approximately 20 per cent. Blohm (7) reviewed the subject, introducing a coefficient of $0.218 \left(D \frac{15^\circ}{15^\circ} \right)$

which he claimed yielded better results than any of those previously suggested, but even this gave errors as high as 13 per cent. Addis and Foster (1) went to the other extreme, claiming that there could be no close correlation between the two variables because the specific gravity factors for the substances found in urine differ so widely.

In Table XII are calculated coefficients for thirteen urines. In this same table, for comparison, are given similarly calculated coefficients for the main urinary substances. It is evident that the average coefficient, $0.228 \left(D \frac{20^\circ}{4^\circ} \right)$ is similar to that of Long $\left(0.260, D \frac{25^\circ}{4^\circ} \right)$ and of Blohm $\left(0.218, D \frac{15^\circ}{15^\circ} \right)$ with variations corresponding to those found by the latter. The coefficients derived from urines of subjects on low protein diets are lower than those from the same subjects on high protein diets. This follows naturally and is due to the much larger amount of urea in the latter cases.

The coefficients in Table XII-B are the reciprocals of the factors in Table XI-B. Converted to these coefficients, the force of Addis' contention is obvious: there is a wide disparity, the inorganic salts as a class having low coefficients, those for urea and creatinine about two to three times as high, with the salts of ammonia giving intermediate values. Nevertheless, the relative constancy of the pattern of the urine constituents on fixed diets indicates that in these cases the concentration of solids can be predicted fairly accurately with coefficients derived from a sample of the group. Although a greater variation in the coefficient might be expected from urines of subjects on uncontrolled diets, the values found with ten random 24-hour specimens have fallen within the extremes of the more carefully controlled series.

DISCUSSION

Since Folin's study (16) of thirty normal urines in 1905, there have appeared in the literature a number of similar but less complete analyses. As a result, the normal range of excretion of the chief urine constituents is fairly well established for American subjects on usual diets. Despite the wide variations in individuals, there is a definite

tendency for this group as a whole to deviate from other groups of different dietary habits such as Europeans (32) with higher urea excretion, Filipinos (11) with low urea and salt outputs, and Japanese (24) with high salt excretion.

The analytical data presented in this paper fall within the normal range as given by other authors and the low and high protein intakes, 40 to 50 grams and 100 to 110 grams respectively represent about the minimum and maximum encountered in the great majority of random 24-hour diets.

There seem to be no recent data where total urine solid excretion has been determined simultaneously with analyses of the major constituents of the urine. The excretion of undetermined organic solids has been either neglected or given as 2 to 3 grams per 24 hours (31). From the available data on the average excretion of the urinary constituents not analyzed in this study, hippuric, citric and oxalic acids, carbohydrate, amino-acids, purine bases, pigments, volatile fatty acids, cystine, etc. could account for only 3 to 4 grams of solid. Hence, the undetermined organic solid of 10 to 15 grams observed in this series of urines is much higher than that previously assumed to be present. The 24-hour output of undetermined solid has also been determined on 10 additional normal adult subjects of both sexes who were allowed a free choice of diet. The values obtained were the same as for the subjects on the controlled diets, 9 to 15 grams. Organic acids, as has been noted, make up a large part of this solid and the significance of these compounds was not apparent to the earlier investigators.

The assumption that the specific gravity of urine can be accounted for on the basis of the concentration of dissolved substances alone, without recourse to physico-chemical phenomena, has been implied in the work of all of those who have calculated the specific gravity contributions of individual substances. Since this has been shown to be a valid assumption the available data may be compared with those of Table VII. Our results for the sum of urea and Cl as NaCl on the high protein diet are in accord with those of Pepper (35) and Ishizu (24) although the data of these investigators show somewhat greater variation in both urea and chloride since they are based on

urine from subjects on unrestricted diets. In all cases however, urea and NaCl account for $\frac{1}{2}$ to $\frac{3}{4}$ of the specific gravity. On the assumption that specific gravity was determined by urinometer at D_{4}^{20} and that the average urine pH was 6.0. Forlin's data (16) may be used to calculate individual specific gravity contributions. The results are almost identical with our high protein data. Urea accounted for an average of 26 per cent, chloride 23 per cent, sulfate 15 per cent and phosphate 16 per cent of the total. In individual urines 72 to 90 per cent of specific gravity was accounted for by these substances. The data of Concepcion (11) for Filipinos on the other hand are comparable with our low protein data. For these groups urea + NaCl account for slightly less than $\frac{1}{2}$ of the specific gravity.

Our data would seem to invalidate the conclusion of Alving and Van Slyke that the concentration test measures chiefly the ability of the kidney to concentrate mineral salts because of their assumption that these comprise $\frac{3}{4}$ of the specific gravity of the urine. Actual measurements show that mineral salts contribute only 50 to 60 per cent of the specific gravity while urea and other organic constituents make up the remainder. Consequently the concentration test measures equally the capacity of the kidney to excrete both inorganic and organic substances. A dilute urine in a normal individual is the result of excretion of larger volumes of water with equal reduction of the gravity contributions of salts and organic substances, the specific gravity patterns remaining the same.

Specific gravity is not the only physical property of urine which may be used as an index of the concentration of dissolved solids. Cryoscopy was introduced in 1897 by von Koranyi (26) and carefully studied by Kövesi and Roth Schultz (27). Since the depression of the freezing point of water is dependent on the number of dissolved particles, rather than their chemical nature, this method might have some advantage over specific gravity as a measure of total solid but ionization of electrolytes is a disturbing factor. Von Hahn (20) has correlated surface tension with specific gravity so that this property might also be used as a measure of concentration. Blohm (7) introduced a refractometric method which is probably the most

rate of any of the physical measures of total solid concentration, and gives values within 5 per cent of those determined by analysis. Its accuracy rests on the fact that, whereas the specific gravity factors of the various urinary constituents range from 0.9 to 3.6 times the factor of urea (concentration expressed in grams per liter) the "refractometric indices" of the same constituents range from 0.8 to 2.0 times that of urea, most of them being 1.1 to 1.4 times greater. Variations in concentrations of individual constituents therefore produce smaller changes in the refractometric index than they do in the specific gravity. Like the cryoscopic method, however, this procedure requires apparatus not commonly available.

All of these attempts to determine total solid output by a simple measurement of some physical property have been devised in order to test the function of the kidney in the excretion of solids. The colligative properties of a solution, vapor pressure, osmotic pressure, and freezing point depression are not directly proportional to solid concentration because of the ionization of electrolytes. The specific gravity test is the simplest and most widely used at present. Despite its widespread use the exact relationship between it and the composition of the urine has never been systematically studied. The results obtained here indicate that specific gravity measures concentration roughly in the same manner as the colligative properties, *i. e.*, with electrolytes having relatively greater effect per unit concentration than the organic substances.

Whether the low specific gravity encountered in kidney disease is simply the result of dilution, with preservation of the normal pattern of specific gravity contributions, or whether there is greater impairment in ability to concentrate some substances than others, must await similar analyses of pathological urines which are in progress.

SUMMARY

1 Evidence is presented that the specific gravity of a complex dilute solution of inorganic and organic substances can be calculated with considerable accuracy. Electrolytes have a greater effect on specific gravity than organic solutes for equivalent concentrations. The effect of electrolytes is approximately a summation of the specific gravity effects of the individual ions.

2 The specific gravity of an artificial solution made up of the major substances commonly found in normal urine is the sum of the specific gravity effects of the individual substances.

3 The specific gravity of urine is a simple additive function of the concentration of its individual solutes.

4 Forty-eight 24-hour urines from 6 subjects with normal renal function have been analyzed for chloride, sulfate, phosphate, bicarbonate, urea, creatinine, pH, and total solids, and the specific gravity contributions of the inorganic salts, urea, and creatinine have been calculated. In urine from subjects on a low (40 to 50 grams) protein diet, urea accounts for 15 to 20 per cent, chlorides for 25 to 30 per cent, sulfate and phosphate together 15 to 25 per cent, bicarbonate 1 to 5 per cent, and creatinine 1 to 2 per cent of the observed specific gravity. On a high (100 to 110 grams) protein diet, urea, sulfate, and phosphate contribute a slightly higher proportion to the specific gravity. On the lower protein diets, 70 to 80 per cent, on the higher protein diets, 80 to 90 per cent of the specific gravity is accounted for by all the substances measured.

5 The undetermined fraction of specific gravity comprises 10 to 30 per cent of the observed value and in the same urines the excretion of undetermined solids amounts to 10 to 15 grams daily or from 10 to 30 per cent of the total solids. The undetermined solid is composed of organic substances. This fraction is relatively low in nitrogen, of small molecular size (dialyzable) and is largely endogenous in origin. Approximately one-half of this fraction is made up of organic acids.

6 The various coefficients proposed for estimation of total solids in urine from specific gravity are valid only for urines of the same relative composition, since the coefficients of organic and inorganic solutes differ by 200 to 300 per cent. Consequently, in normal subjects on fixed diets, the solid content can be estimated quite accurately from specific gravity by means of coefficients. In random urines, on the other hand, the variation in composition is sufficient to make the use of coefficients too inaccurate for exact analytical use.

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IODINE COMPONENTS OF THE BLOOD CIRCULATING THYROGLOBULIN IN NORMAL PERSONS AND IN PERSONS WITH THYROID DISEASE

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It is now well established that the amount of iodine circulating in the blood is roughly an index of thyroid activity. Iodine values below 5 gamma per cent are usually found in myxedema, and values over 10 gamma per cent are suggestive of hyperthyroidism. A portion of the blood iodine is inorganic and presumably inert in a hormonal sense the remainder is organic and probably represents the circulating hormone or its components. Very little is known of the nature of this organic iodine. In the course of experiments on the production of antibodies to human thyroglobulin, it seemed that antiserum potent with respect to thyroglobulin antibodies might be used to detect thyroglobulin in human serum by appropriate immunologic reactions.

METHOD

The technique used to obtain antithyroglobulin serum is similar to that of Hektorn and Schulhof (1) Rosen and Marine (2) and Schulhof (3). Thyroglobulin was prepared from human thyroid glands which had been removed at operation and made relatively free from serum.¹ It was kept in suspension at its isoelectric point. The protein content was usually 1.5 to 2.0 per cent, and the iodine about 5 to 6 mgm. per cent. This material was injected into rabbits intraperitoneally intravenously or into subcutaneous nodules according to the method described by Dienes (4). The injections were given for 2 to 3 days in succession, with rest periods of 4 to 6 days. The intraperitoneal injection contained about 75 to 150 mgm. of thyroglobulin, the intravenous injection 15 to 20 mgm. and the intranodular injection 1 to 3 mgm. for each nodule. After a period of 4 to 8 weeks, the serum of such animals usually contained sufficient antibodies to be of value in testing for very small amounts of thyroglobulin. In some instances, animals were injected for several months in succession in order to increase their antibody titer. The rabbit antiserum contained not only antibodies for human thyroglobulin but also small amounts of antibodies for human serum protein. Consequently

before using such serum in immunologic tests it was necessary to absorb the antibodies against human serum. This was done by mixing the antiserum with human serum in the proportion of 1 to 0.25 incubating the mixture overnight, and centrifuging the small precipitate formed. A similar absorption technique was used by Stokinger and Heidelberger (5).

The presence of antibodies was determined by the ring precipitin test, using the undiluted rabbit serum against dilutions of thyroglobulin or human serum. Thyroglobulin was first dissolved in dilute alkali at a pH of 8.0 to 9.0 until it was almost clear and the insoluble portion removed by centrifugation. Normal saline used in making dilutions was also adjusted to the same pH. Otherwise, at the usual pH of normal saline there would be precipitation of thyroglobulin.² The antiserum was introduced at the bottom of small tubes and the dilutions of thyroglobulin or human serum were layered on top. The results were read after 1 to 2 hours at room temperature.

In attempting to evaluate the potency of the antiserum the precipitation test and complement fixation technique were used to confirm the results of the precipitin ring test. However in detecting thyroglobulin in human serum, only the precipitin test was used.

Blood for tests was obtained from normal people, from patients with myxedema from thyrotoxic patients before and after iodization and after operation, and directly from the thyroid veins during operation. In all, about 66 samples of blood were tested. Four were from normal people, 2 from patients with myxedema, 15 from thyrotoxic patients before iodization 2 from thyrotoxic patients after iodization, 22 from the thyroid veins of toxic and non toxic goiter patients obtained during the course of operation, and 21 from goiter patients after operation. Numerous normal bloods were used as negative controls when the blood from thyroid veins was tested for thyroglobulin. Some of the blood samples were tested with more than one antiserum. In addition the 24-hour urine specimens of 3 hyperthyroid patients were concentrated according to the method used by Rawson and Starr (6) in concentrating thyrotropic hormones and tested for the presence of thyroglobulin.

¹ I am indebted to Dr W T Salter and Dr J M. Muns of the Boston City Hospital for the preparation of thyroglobulin.

² Undoubtedly some of the thyroglobulin is denatured in the process of preparation and on standing but the amount is seldom more than 10 per cent. The precipitate formed when normal saline is added is probably due to this fraction of thyroglobulin.

RESULTS

The antisera used in these experiments usually were able to detect thyroglobulin in dilutions up to 1 10,000 or 1 20,000 of a 15 per cent solution. In other words, the amount of thyroglobulin that could be detected was 0.08 to 0.15 mgm per 100 cc of serum or $\frac{1}{4}$ to $\frac{1}{2}$ gamma per cent of thyroglobulin iodine, an amount practically negligible. Even the less potent sera could detect 1 gamma per cent of thyroglobulin iodine. Table I represents the result of a typical pre-

TABLE I

The precipitin test for thyroglobulin in blood of a hyperthyroid patient (J T Number 4914) before and after absorption of rabbit antiserum by human serum

	Dilutions of human serum					
	1 2	1 4	1 8	1 16	1 32	1 64
Rabbit Number 2K antiserum	↓↓+	↓++	↓+	++	++	+
Same antiserum incubated with human serum	±	—	—	—	—	—

↓ indicates precipitation + indicates ring precipitin

cipitin test for thyroglobulin in the blood of an exophthalmic goiter patient. The unmodified rabbit antiserum gave strongly positive reactions, as indicated in the second horizontal column, but the absorbed serum gave negative reactions, as shown in the last horizontal column. The positive reactions were obviously due to antibodies against human serum. This particular antiserum was able to detect about 0.1 mgm of thyroglobulin per 100 cc of blood.

With the precipitin test no detectable amount of thyroglobulin was discovered either in the various normal human sera, in the sera of patients with myxedema, or in the sera of thyrotoxic patients before and during iodization. The patients with hyperthyroidism were unselected and therefore represent all grades of severity, the metabolic levels ranging from plus 27 to plus 75. Similarly, no thyroglobulin was detected in the urine of 3 thyrotoxic patients.

In spite of the absence of any appreciable amount of thyroglobulin in the blood, there still remained the possibility that thyroglobulin was secreted unchanged directly into the thyroid veins,

as reported by Carlson, Hektoen and Schulhof (7) in dogs. Consequently, blood was obtained directly from the thyroid veins during the course of thyroidectomy, attempt being made to get samples both at the beginning and at the end of operation. In most instances, samples were also obtained from the peripheral blood 2 to 4 hours and 24 to 36 hours after operation. In all, 9 cases of exophthalmic goiter and 4 cases of non-toxic goiter were studied in this fashion. In 5 additional cases blood was obtained only in the post-operative period. Where thyroglobulin was present in a blood sample, the attempt was made to determine the quantity by setting up simultaneous precipitin tests with a known solution of thyroglobulin. By comparing the known and unknown tubes, an approximate estimate of the amount present in the unknown was obtained. Table II shows a typical example of precipitin tests for thyroglobulin in blood from thyroid veins and in peripheral blood. Table III summarizes the results of the precipitin tests on all samples of blood taken during and after operation.

In only 2 of the 12 samples of blood taken from the thyroid vein at the beginning of operation was there any evidence of thyroglobulin. This amounted to 0.1 mgm and 1.6 mgm per 100 cc, respectively. In 1 of these cases, (C L), a second specimen from the opposite thyroid lobe showed a slight increase in thyroglobulin. In the other, (D K), the second specimen was missed so that one cannot tell whether there was an increase or not. In this case there was no thyroglobulin detected in the peripheral blood 2 hours after operation. In a third case, (E Y), the first thyroid vein sample was obtained during the course of a second hemithyroidectomy. It is therefore listed as a second specimen. Two patients showed traces (±) of thyroglobulin in the first thyroid vein sample, but such evidence is doubtful and must be classified with the negative results.

Of the 8 thyroid vein samples obtained during or at the end of the second stage of subtotal thyroidectomy, 7 showed appreciable amounts of thyroglobulin, varying from 0.2 to 13.0 mgm per 100 cc. In 5 of these, the first thyroid vein sample taken at the beginning of operation was negative. In only one subtotal thyroidectomy,

TABLE II

The precipitin test for thyroglobulin in blood of a hyperthyroid patient (C.L. Number 218911) compared to a known thyroglobulin solution

	Dilutions of human thyroglobulin					
	1:1 000	1:2 000	1:4 000	1:8 000	1:16 000	1:32 000
Rabbit antiserum incubated with human serum	↓+	++	+ or ++	+	+	±
	Dilutions of patient's sera					Blood sample number
	1:1	1:2	1:4	1:8	1:16	
Rabbit antiserum incubated with human serum	+	±	—	—	—	1 From right middle thyroid vein beginning of operation.
	++	+	±	—	—	2 From left inferior thyroid vein end of operation
	+ or ++	+	—	—	—	3 Peripheral blood 3 hours post-operative
	—	—	—	—	—	4 Peripheral blood 24 hours post-operative.

TABLE III

Results of precipitin tests for thyroglobulin in bloods taken from thyroid veins during operation and from peripheral blood after operation (absorbed serum used)

HYPERTHYROID PATIENTS					
Case	Right middle thyroid vein	Left inferior thyroid vein	2-4 hours post operative	24-36 hours post operative	Postoperative reaction
M.L.	0	0			mild
A.C.	0	2			mild
R.B.	±	16		1 or ±	severe
M.LaF*	±	±	0	0	moderate
D.K.	16		0	0	moderate
E.Y†		8	±		moderate
C.L.	1	2	2	0	mild
I.C.	0	128	2	2	mild
I.R.	0	4	2		moderate
A.T.			0	0	mild
R.L.G.			0	0	mild
M.M.				0	mild
E.C.				0	mild
B.P.				0	thyroid crisis
NON TOXIC GOITER PATIENTS					
A.B.	0				mild
E.M.	0	8		0†	mild
E.G.*	0	0		0†	mild
S.Y.	0		0		mild

The values indicate the highest dilution of human serum which gives a positive precipitin test ± represents an amount less than 1

* Hemithyroidectomy

† Second hemithyroidectomy

‡ 12 hours postoperative.

(M L.), were both thyroid vein samples negative for thyroglobulin. In 2 other cases, (M LaF and F G) the operative procedure was that of initial hemithyroidectomy. Here, the second thyroid vein sample was obtained at the end of hemithyroidectomy but from the unoperated side. The latter received very little manipulation before the blood was drawn. In both, the blood was free of thyroglobulin at the beginning and at the end of operation. These results suggest that the operative procedure of cutting and manipulating the gland caused the extrusion of thyroglobulin into the blood. As indicated before the blood of 2 thyrotoxic patients was tested for the first time postoperatively at 4 hours and at 24 hours, and was found to be free of detectable thyroglobulin. The blood of 2 others was negative 24 hours after operation. The blood of a fifth patient was examined 36 hours after operation during a thyrotoxic crisis and no thyroglobulin was found. Thus of the 9 samples of blood examined 2 to 4 hours after operation 3 showed small amounts of thyroglobulin of the 12 samples examined 12 to 36 hours postoperatively 1 showed a small amount and another showed a possible trace of thyroglobulin. In general, the thyroglobulin that gets into the blood stream during operation tends to diminish or to disappear altogether

It might be supposed that the amount of thyroglobulin present in the blood during and after operation would condition the severity of the postoperative reaction. This was not the case. As indicated in the next to the last column of Table III, the severity of the postoperative reaction had no relation to the amount of thyroglobulin found in the thyroid veins during operation or in the peripheral blood after operation. The patient, (I C), who had the most thyroglobulin in the thyroid veins during operation had a mild postoperative reaction. This patient was 1 of the 2 patients who still showed thyroglobulin in the blood after 24 hours. Moreover, the 1 patient with a "thyrotoxic crisis" did not have any thyroglobulin during this episode. Unfortunately, blood from the thyroid vein was not obtained in this case. The only other severe reaction was shown by a patient, (R B), whose thyroid veins contained a moderate amount of thyroglobulin. However, this patient's gland was friable and bled easily so that the technical procedure was difficult and of more than average duration.

DISCUSSION

The absence of any appreciable amount of thyroglobulin in normal human blood is in agreement with the findings of Hektoen, Carlson and Schulhof (8) in goitrous dogs. One might have expected that in hyperthyroidism, which is characterized by a tremendous outpouring of thyroid hormone and an increase in blood iodine, there would be detectable amounts of thyroglobulin in the circulation. Hektoen and Schulhof (9) failed to find thyroglobulin in the blood of a small number of exophthalmic goiter patients. The absence of thyroglobulin in the peripheral blood in hyperthyroidism at various stages of the disease, as indicated above, is confirmatory. It proves that the excess organic iodine in hyperthyroid blood is not due to thyroglobulin. What it is due to I am unable to say on the basis of the present experiments.

The finding of appreciable amounts of thyroglobulin in thyroid vein blood and in thyroid lymph of dogs by Carlson, Hektoen and Schulhof (7) would lead one to believe that most of the thyroid hormone is secreted directly into the blood capillaries and lymphatics as thyroglobulin. The

absence of thyroglobulin in the general circulation of these dogs may be due either to rapid dilution by the blood, rapid absorption of thyroglobulin by the tissues or rapid digestion of the protein molecule into smaller components. However, it would be difficult to explain their inability to find thyroglobulin in the thoracic duct. The question naturally arises, could their finding of thyroglobulin in the thyroid veins and lymphatics be explained on the basis of trauma? These investigators themselves were unable to exclude possible injury to the thyroid cells.

In humans, Hektoen and Schulhof (9) report the finding of thyroglobulin in 5 of "a considerable number" of thyroid vein blood samples. Unfortunately, no information is available regarding the stage of operation at which these bloods were obtained. In goiter operations on humans it is almost impossible to avoid some degree of trauma in exposing the blood supply of the gland, whether the goiter is toxic or non-toxic. Consequently, the results of Hektoen and Schulhof and the above reported absence of detectable amounts of thyroglobulin in 10 of the 12 thyroid vein samples at the beginning of operation are significant. They immediately suggest that the positive results are due to artificial factors, namely trauma. The finding of thyroglobulin in 7 of 8 thyroid vein samples taken in the later stages of subtotal thyroidectomy supports this viewpoint. Between the first and second thyroid vein samples considerable manipulation and cutting of the gland had taken place. In short, colloid was squeezed into the circulation but not secreted into it. It must therefore be concluded that neither in the normal gland nor in the hyperplastic gland does thyroglobulin enter the blood from the follicle as such, but must be broken down into smaller components.

The anatomic mechanism for release of thyroid has recently been studied by Williams (10). He observed living thyroid follicles in chambers implanted in rabbits' ears. He describes a process by which a droplet of colloid is pinched off and comes to lie within the follicular wall. The droplet slowly decreases in size until it disappears. It is never extruded into the interfollicular spaces. In other follicles a whole section of wall may be compressed for a short time. It

is supposed that colloid diffuses through this thin area in the follicle. Williams maintains that the follicular wall remains intact. If this is the case, one may logically assume that the thin portion of the follicle acts as a living semi permeable membrane and that a large molecule like thyroglobulin could hardly diffuse through it. It would seem more likely that thyroglobulin is first digested and that the resultant small fragments then diffuse through the follicle wall. Thus the uncommon occurrence of thyroglobulin in thyroid veins is consistent with the anatomic observation of Williams.

It is of interest to speculate on the role of thyroglobulin in metabolic processes. Is thyroglobulin merely the protein storehouse for smaller and hormonally active components? The failure of the thyroid follicles to release thyroglobulin as such and the circulation in the blood of fragments of thyroglobulin rather than the parent substance would suggest that thyroglobulin is not the metabolic hormone. Thyroxine or thyroxine like substances must be the true accelerators of oxidative processes. However the possibility remains that these fragments are resynthesized into thyroglobulin when they reach the tissues and that hormonal action is after all dependent upon the formation of thyroglobulin.

SUMMARY AND CONCLUSION

Rabbits injected with human thyroglobulin produced antiserum which was able to detect by precipitin reaction minute amounts of thyroglobulin in solution, namely 0.08 to 0.15 mgm. per 100 cc. By means of this reaction no detectable amounts of thyroglobulin were discovered in the blood of numerous normal patients, 2 myxedematous patients, 15 thyrotoxic patients before iodination and 2 thyrotoxic patients after iodination. No thyroglobulin was detected in the urine of 3 hyperthyroid patients.

It is therefore concluded that the excess iodine usually present in the blood of hyperthyroid patients is not due to circulating thyroglobulin.

The precipitin reaction was applied to venous blood from the thyroid obtained at various stages of thyroidectomy in 9 cases of exophthalmic goiter and in 4 cases of non toxic goiter. Ten of the

12 thyroid vein samples obtained at the beginning of operation were negative, 2 thyroid vein samples obtained at the end of hemithyroidectomy but from the unoperated side, were also negative. On the other hand 7 of 8 thyroid vein samples obtained during or at the end of the second stage of subtotal thyroidectomy showed appreciable amounts of thyroglobulin. The thyroid vein blood of 5 of these cases was negative at the beginning of operation. These results suggest that the presence of thyroglobulin in the blood during and after operation is due to the extrusion of colloid into the circulation by trauma to the gland and that under ordinary conditions thyroglobulin does not leave the follicles normal or hyperplastic, as such. This deduction is consistent with the observations of Williams on the release of colloid from living thyroid follicles.

The thyroglobulin that gets into the circulation during operation is either rapidly destroyed or fixed by the tissues. Of the 12 blood samples obtained 12 to 36 hours after operation 10 showed no thyroglobulin, 1 showed 0.2 mgm. per cent, a decrease from 13.0 mgm. per cent, and 1 showed a possible trace, a decrease from 1.6 mgm. per cent.

The presence or absence, or the amount of thyroglobulin in the blood during and after operation does not correlate with the degree of postoperative reaction. This fact does not exclude the possibility that absorption of hydrolyzed products of thyroglobulin during or immediately after operation plays an important role in the development of thyrotoxic 'crises'.

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THE EFFECT OF THE APPLICATION OF TOURNIQUETS ON THE HEMODYNAMICS OF THE CIRCULATION

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The treatment of acute left ventricular failure by morphine and venesection frequently produces as dramatic relief of symptoms as does the intravenous administration of glucose in a case of insulin shock. Cases which have not responded to morphine alone may recover promptly after the removal of from 500 to 800 cc. of blood.

Many clinicians (1, 2, 3, 4) believe that pooling of blood in the extremities by means of tourniquets is as effective as phlebotomy in the treatment of left ventricular failure. While this is a widespread clinical impression, experiments demonstrating that a significant quantity of blood is pooled in the extremities by venous tourniquets are lacking. Without this knowledge there is no rational basis for comparing the efficacy of these two forms of therapy. The purpose of this study is, therefore, to measure the amount of blood pooled in the extremities by venous tourniquets and to determine whether this amount is sufficiently large to be of clinical significance. The dye method was used for the determination of the blood volume, and tourniquets were used to separate the blood circulating in the extremities from that of the remainder of the body.

METHOD

Each of the following 3 experiments was performed on 4 normal males and 1 subject with chronic arthritis.

Basal blood volume. The total blood volume was calculated from the proportion of plasma to cells, as shown by hematocrit determinations. The plasma volume was determined by the dye method of Gibson and Evans (5) as adapted to the photoelectric microcolorimeter by Gibson and Evelyn (6).

The blood volume of head, trunk, and arm at rest. On another day the arterial circulation to the extremities was occluded by inflating tourniquets on the right arm and both thighs to a pressure of 250 mm. of Hg. As the pressure was thrown into the cuffs from a large reservoir the occlusion was rapid. Ten mgm. of dye were immediately injected into the left antecubital vein. Ten minutes after the injection of the dye, a needle was placed in the left antecubital vein and from 4 to 6 sam-

ples of blood were taken during the next 10 minutes. The amount of blood in head, trunk, and arm at rest was determined from the concentration of the dye in these samples and from the hematocrit. Twenty minutes after the injection of the dye the tourniquets were released. Samples of blood were taken for 10 to 50 minutes after the release of the tourniquets.

Blood volume of head, trunk, and arm after venous congestion of the extremities. On a third day the three extremities were congested by inflating tourniquets on their proximal portions to diastolic pressure. At the end of 7 to 10 minutes the arterial circulation to these extremities was cut off from the remainder of the body by raising the pressure in the cuffs to 250 mm. of Hg. Ten mgm. of dye were then injected into the left antecubital vein. Ten minutes later a needle was placed in the left antecubital vein and from 4 to 6 samples of blood were taken during the next 10 minutes. The blood volume of head, trunk, and arm was determined from the concentration of the dye in these samples and from the hematocrit. Twenty minutes after the injection of the dye the tourniquets were released. Samples of blood were taken for 20 to 60 minutes after the release of the tourniquets.

The volume of blood normally present in the three extremities was obtained by subtracting the result obtained in experiment 2 from that obtained in experiment 1 and the volume of blood that was removed from head, trunk, and arm by venous congestion was obtained by subtracting the result obtained in experiment 3 from that obtained in experiment 2.

In all the experiments the subjects had fasted for 12 hours. They rested comfortably on a bed for 30 minutes before the experiments started. The room temperature was from 20 to 22 C. In the experiments measuring volume of blood in head, trunk, and arm, the vessels of the extremities were neither contracted by cold nor dilated by heat. In 3 experiments with venous congestion the extremities were heated by immersion in warm water before the tourniquets were applied. However, as warming produced no demonstrable difference in the amount of blood pooled, it was discontinued.

To secure the maximum pooling of blood in the extremities with minimum discomfort to the subject, the cuffs on the thighs should be at least 12 cm. wide and the cloth should be long enough to wrap around the thigh several times. The tourniquets on the extremities were placed as close to the body as possible. Padding was placed under the distal portion of the cuffs to pre-

vent them from sliding down the thighs when they were inflated. Subjects with long, thin limbs were the most suitable.

In addition to the blood volume determinations, the subjective sensations, skin color, and respiratory rates of the subjects were noted. The effects of venous tourniquets were also observed in 2 other normal subjects in whom blood volume determinations were not done. In 2 hypertensive subjects the effect of a more prolonged venous occlusion on arterial pressure and heart sounds was studied.

RESULTS

Basal blood volume In 5 subjects (Table I) the basal blood volume ranged from 5010 to 6100 cc of blood, with an average of 5580 cc.

Arterial tourniquets In the same 5 subjects (Table I) the volume of blood circulating in the head, trunk and one arm, when the circulation to the other three extremities had been occluded without venous congestion, ranged from 4040 to

TABLE I
The amount of blood in the head, trunk and arm

Subject	Age of subject	Height	Weight	Basal blood volume			Volume of blood in head trunk, arm without congestion			Volume of blood in head trunk, arm with venous congestion			Volume of blood in three extremities without congestion	Decrease in volume of head, trunk, arm with venous congestion
				Plasma volume	Total blood volume	Hematocrit	Plasma volume	Total blood volume	Hematocrit	Plasma volume	Total blood volume	Hematocrit		
JW	24	73	143	3490	6100	42.9	2900	5190	44.1	2490	4300	42.1	910	890
LT*	42	71	152	2750	5010	45.1*	2220	4380	49.3*	1950	3800	48.7*	630	580
RE	27	69	143	2950	5220	43.5	2280	4040	43.5	1850	3300	44.0	1180	740
JR	31	73	176	3380	5970	43.4	2930	5080	42.4	2480	4300	42.3	890	780
HS	28	70	170	3080	5600	45.0	2510	4700	46.6	2180	4080	46.6	900	620

* This subject had chronic arthritis. His hematocrit showed a slight fall during his stay in the hospital.

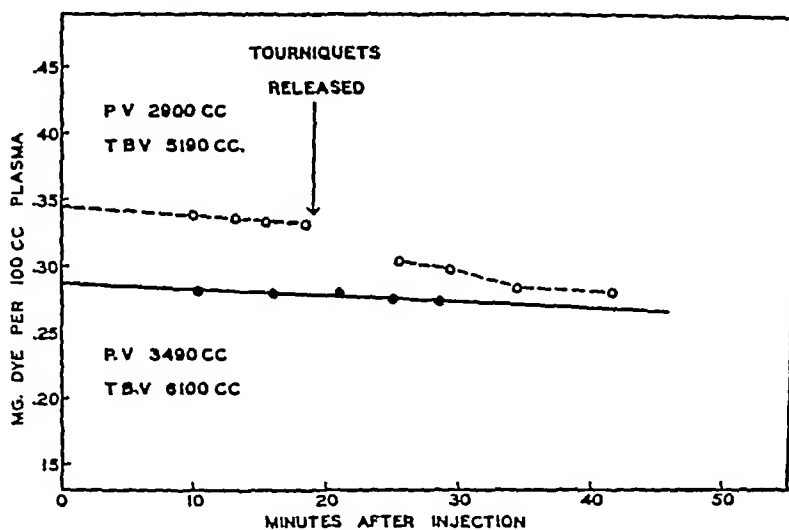


FIG 1 AMOUNT OF BLOOD NORMALLY PRESENT AT REST IN ONE UPPER AND TWO LOWER EXTREMITIES (SUBJECT J W)

Solid line represents concentration of dye (Evans blue) in plasma after the injection of 10 mgm of dye. On the basis of this curve and the hematocrit, the basal blood volume is 6100 cc. The first portion of the broken line represents the concentration of dye in the plasma of head, trunk, and arm after injection of 10 mgm of dye. The volume of blood in this portion of the body is 5190 cc. The lower portion of broken line represents the concentration of dye in the plasma after release of tourniquets. Note that plasma volume is decreased after the release of tourniquets and has not completely returned to normal at the end of 20 minutes.

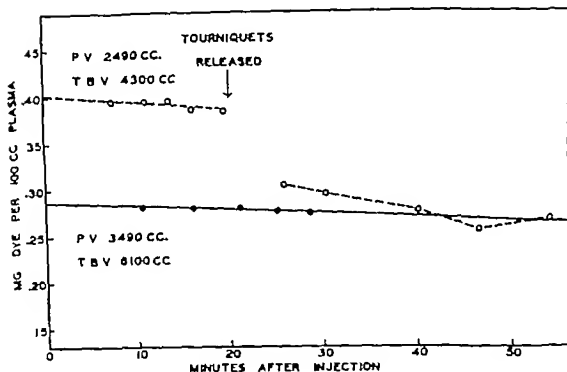


FIG 2. AMOUNT OF BLOOD WHICH IS REMOVED FROM HEAD TRUNK AND ARM BY VENOUS TOURNIQUETS (SAME SUBJECT AS IN FIGURE 1)

The basal blood volume (solid line) is 6100 cc. The first portion of the broken line represents concentration of dye in plasma of head, trunk and arm when the extremities are congested. The volume of blood in this portion of the body is 4300 cc. When this volume (4300 cc.) is subtracted from the normal volume of the head trunk and arm (5190 cc.) as determined in Figure 1 it is found that 890 cc. of blood are removed by congesting the three extremities. The lower portion of the broken line represents the concentration of dye in the plasma after the release of tourniquets.

5190 cc. with an average of 4680 cc. The amount of blood present at rest in the right arm and lower extremities ranged from 630 to 1180 cc with an average of 900 cc or 16 per cent of the total blood volume. A typical experiment is shown in Figure 1.

After the tourniquets were released the plasma volume remained lower than the basal volume for 20 to 30 minutes. This was presumably the result of an increase in permeability of the capillaries of the extremities from the prolonged arterial occlusion. In the experiments with venous tourniquets given below the combined effect of increased venous pressure and anoxia usually resulted in an even greater loss of fluid into the tissues of the extremities.

Subjectively all 5 persons were conscious of discomfort when the pressure was applied but after a few minutes the pain subsided. The extremities felt cool to the subject. Sensation and motor power were greatly diminished in the extremities by the end of 20 minutes. There were no other symptoms, the subjects did not develop nausea, sweating or pallor. After release of the

tourniquets tingling and pain in the extremities were present for several minutes.

Venous tourniquets. In the same 5 subjects (Table I) the volume of blood present in head, trunk and one arm after the other three extremities were congested ranged from 3300 to 4300 cc. with an average of 3960 cc. The congested extremities therefore contained from 1210 to 1920 cc of blood with an average of 1640 cc. (29 per cent of the total blood volume). A typical experiment is shown in Figure 2.

When the three extremities were congested by venous tourniquets the volume of blood circulating in the head, trunk and one arm was decreased by 580 to 890 cc. of blood (average decrease 720 cc.) The volume of blood normally present at rest in the head, trunk and one arm was thereby decreased approximately 15 per cent by pooling blood in the extremities.

The application of venous tourniquets at diastolic pressure produced symptoms of shock in 4 of 7 subjects tested. These symptoms were nausea, sweating and pallor. Blood pressure readings were not made as a tourniquet was on

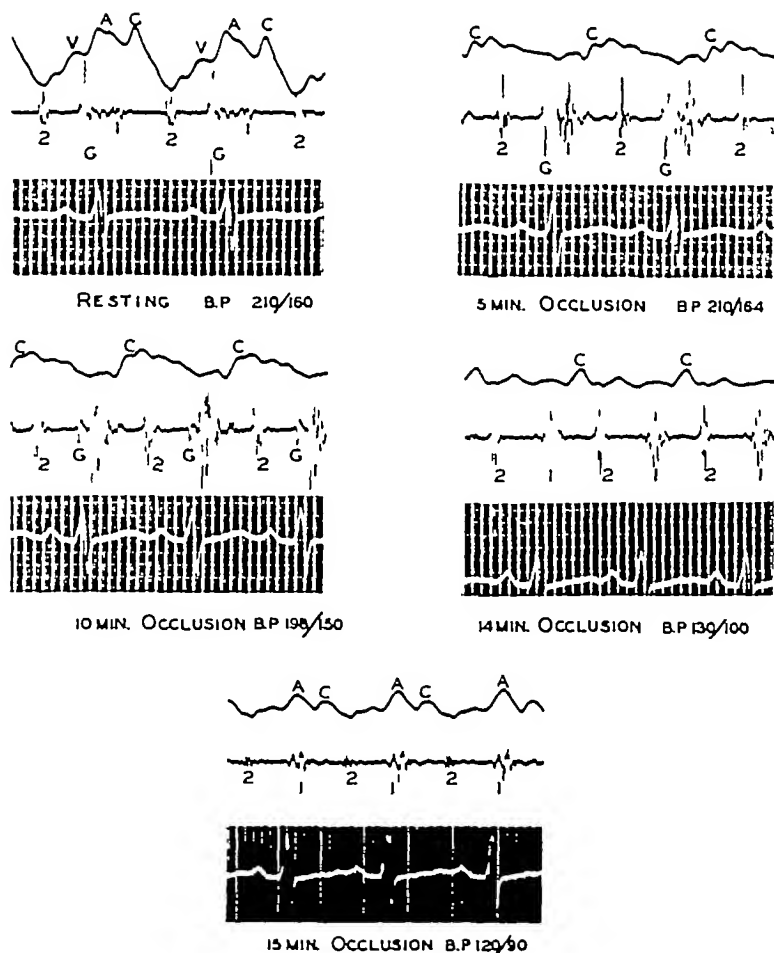


FIG 3 THE EFFECT OF VENOUS TOURNIQUETS (110 MM OF HG) ON THE ARTERIAL PRESSURE AND HEART SOUNDS OF A HYPERTENSIVE SUBJECT, F C

The pooling of blood in the extremities lowers the pulmonary venous pressure and the gallop (g) disappears

one arm and a needle in the vein of the other arm. As these same subjects had experienced no generalized reaction to rapid arterial occlusion, a procedure which was as painful as the venous congestion, it was believed that the symptoms of shock were produced by the rapid pooling of blood in the extremities. After release of the tourniquets the subjects experienced more severe tingling than after the simple arterial occlusion. They also showed distinct hyperpnea. Two of the 7 subjects showed a few petechial spots.

Effect of venous tourniquets in hypertensive subjects In 2 subjects with arterial hyperten-

sion, tourniquets inflated to a pressure of 110 mm of Hg were applied to the three extremities. The resting arterial pressures in these subjects were 210 and 150 mm systolic, and 160 and 110 mm diastolic, respectively. In the first subject sweating, pallor, nausea and mental confusion developed, and at the end of 16 minutes the arterial pressure had fallen to 90 mm systolic and 70 mm diastolic. A marked gallop rhythm (Figure 3), which was present before the tourniquets were applied, had disappeared. On release of the tourniquets the pressure rose rapidly and the gallop gradually returned (Figure 4). In a sec-

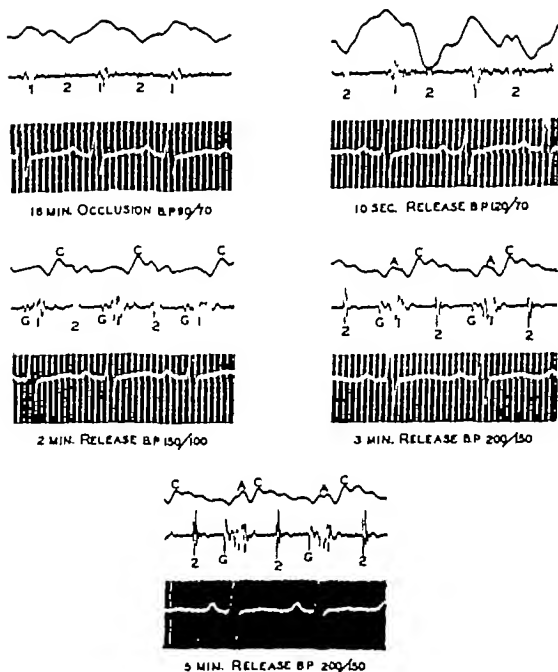


FIG. 4 THE EFFECT OF THE RELEASE OF VENOUS TOURNIQUETS ON THE ARTERIAL PRESSURE AND HEART SOUNDS IN THE SAME SUBJECT

The arterial pressure rises rapidly. The gallop reappears as the pulmonary venous pressure increases.

and subject a similar fall in arterial pressure with symptoms of shock occurred.

DISCUSSION

Venesection in the treatment of acute left ventricular failure has had a sound physiological basis since Blumgart and Weiss demonstrated that the amount of blood present in the lungs is increased in left ventricular failure. These investigators (7) found that the average velocity of the blood in the lungs in these cases was slower than in normal subjects. In the absence of a striking diminution in cardiac output, this decrease in velocity could only result from an increase in the

size of the cross section area of the pulmonary bed. The volume of blood filling this dilated bed must therefore, be greater than in normal subjects. This increase in volume of blood in the pulmonary bed in acute left ventricular failure is the result of temporary imbalance in the function of the right and left ventricles so that the right ventricle brings more blood to the lung than the left ventricle can remove (8). The removal of blood from the body by phlebotomy decreases the amount of blood contained in the engorged lungs and hence relieves the symptoms.

Few investigators have studied the effect of tourniquets on the circulation although clinicians

have long recommended them as a substitute for phlebotomy. Tabora (1) in 1910 showed that venous tourniquets applied to the extremities caused relief of symptoms and a decrease in venous pressure in patients with congestive failure. Fuchs (9) produced symptoms of shock in 5 of 7 subjects studied by this method. The venous pressure decreased moderately in the 2 subjects who did not develop collapse, it was not measured in the others. Brams and Golden (10) found that there was no significant change in blood pressure, pulse rate or venous pressure after the application of venous tourniquets to the extremities of patients with congestive failure and therefore concluded that the procedure had little value. Hamilton and Morgan (11) studied the effect on the vital capacity of the application of venous tourniquets to four extremities in normal subjects. They made observations on the subjects in the dorsal recumbent and standing positions. With tourniquets the vital capacity was definitely increased in the dorsal recumbent position, it was little changed in the standing position. They interpret these data as suggesting that the amount of blood in the lungs can be decreased in normal subjects by pooling blood in the extremities. Jarisch and Gaisbock (12) found that, when the circulation to both legs and one arm was occluded rapidly, the cardiac output decreased because the normal venous return from the extremities was occluded. If the arterial occlusion was prolonged for 15 or 20 minutes the cardiac output rose.

The results of this study show that in normal subjects the volume of blood circulating in the head, trunk and arm at rest is decreased by 580 to 890 cc. by placing venous tourniquets on three extremities. This is more effective than the removal of a corresponding amount of blood by phlebotomy because in the first case the blood is removed from the head, trunk and arm volume, and in the latter instance it is removed from the total blood volume. It is significant that a measurable amount of fluid is lost from the blood stream during the period of venous engorgement. This is in part the result of the increased capillary pressure (13). More fluid would have been lost if the period of congestion had been prolonged. This loss of fluid from the blood stream explains in part the clinical observation that in the treat-

ment of acute left ventricular failure the beneficial effects of tourniquets persist for some time after their release.

In the treatment of congestive failure more dramatic results are obtained in those subjects in whom failure of the left ventricle predominates. When marked failure of the right ventricle is present, the veins are already distended. In addition, when the tissues are tight with edema fluid it is more difficult to pool blood in the extremities because the venous bed is less distensible.

The appearance of the symptoms of vascular collapse after venous tourniquets in half the group of normal subjects is further proof that a large amount of blood is removed from the head, trunk and arm. More blood can probably be pooled in hypertensive than in normal subjects because the venous tourniquets can be inflated to higher levels without interfering with the arterial inflow to the extremities. In the 2 hypertensive subjects studied, profound collapse occurred, the systolic pressure falling from 210 and 150 mm. to 90 and 100 mm., respectively. While venous pressures were not taken in these experiments, the venous pulsations in the neck decreased and the veins of the free extremity appeared empty and collapsed. In addition to the fall in peripheral venous pressure, the pulmonary venous pressure was probably lowered in the case of F. C. This subject had early left ventricular failure and a marked mid-diastolic gallop. The gallop occurred at the time of rapid ventricular filling and was greatly accentuated by exercise. It was a manifestation of the greater than normal pressure differential between the auricle and ventricle in early diastole which caused a rapid flow of blood into the ventricle and produced a sound. When the venous tourniquets were applied, the gallop disappeared as blood was pooled in the extremities because the volume of blood and the venous pressure in the lungs were decreased. On release of the tourniquets the blood pressure returned to normal and the gallop slowly reappeared. The gallop was not present during the period of hyperpnea which occurred 10 to 15 seconds after the release of the tourniquets. This indicates that the increase in respirations is not caused by pulmonary congestion from the sudden release of the tourniquets. If the pulmonary venous pressure had been sud-

denly increased by this mechanism, the gallop would have been present. The hyperpnea probably results from chemical stimulation of the respiratory center as the blood with a lowered pH returns from the extremities.

SUMMARY AND CONCLUSIONS

1 In 5 subjects (4 normal and 1 with chronic arthritis) the basal blood volume averaged 5580 cc. The blood volume of the head, the trunk, and one arm averaged 4680 cc. Therefore, the average amount of blood in one upper and two lower extremities was 900 cc., or approximately 16 per cent of the total blood volume.

2. In the same 5 subjects an average of 720 cc of blood was removed from head, trunk and arm by placing venous tourniquets at diastolic pressure on three extremities. This represented 15 per cent of the volume of blood normally circulating in the head, trunk and arm

3 In 4 of 7 normal subjects tested, sufficient blood was pooled in the extremities to produce symptoms of collapse, i.e. nausea, sweating and pallor. In 2 hypertensive subjects the venous tourniquets produced a marked fall in arterial pressure and profound collapse.

4 In 1 hypertensive subject pooling of blood in the extremities caused the disappearance of a marked diastolic gallop. This indicated that the tourniquets were effective in lowering the pulmonary venous pressure

5 When tourniquets were applied to the extremities the plasma volume was lowered by transudation of fluid into the tissues. Thus the beneficial effect of the tourniquets persists in part for some time after release.

6 This investigation demonstrated that as much blood was removed from the general circulation by venous tourniquets as by the usual phlebotomy. It presents a rational basis for this method of treatment of left ventricular failure.

The authors wish to express their appreciation to Dr. Soma Weiss for helpful guidance and criticism in this

work. This investigation was carried out with the technical assistance of Miss Blanche Curtis and Miss Evelyn Berstein.

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ON THE EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

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In order to gain a better understanding of the role of infection in chronic glomerulonephritis, a study of the exacerbations in this disease was undertaken. Sixty-eight patients with chronic glomerulonephritis were observed closely for periods of from 1 to 8 years. An analysis was made of the incidence of the exacerbations in this group, the presence and nature of the infection preceding each exacerbation, the latent period between the infection and the signs of increased renal inflammation and finally the immediate and subsequent effect of each exacerbation on the renal function. It was hoped that these observations might contribute information concerning factors responsible for the progressive nature of chronic Bright's disease.

Definition of exacerbation in chronic glomerulonephritis

Although there is agreement as to the criteria necessary to establish the diagnosis of acute glomerulonephritis, it is difficult to derive a satisfactory definition for the exacerbation in the chronic phase of this disease. Criteria to describe this state are of necessity arbitrary. For the purpose of this investigation we have been guided by the following objective and relatively simple considerations:

It has been assumed that the development of an exacerbation in chronic glomerulonephritis is indicated by an abrupt and marked increase in the degree of hematuria. A concomitant impairment of renal function adds weight to this interpretation but its absence does not militate against the diagnosis of exacerbation. Since most patients with chronic glomerulonephritis usually pass moderate to large quantities of albumin in the urine, changes in this abnormality are difficult to interpret. Slight variations in the urinary

output of erythrocytes, a common finding in chronic glomerulonephritis, do not necessarily signify the presence of an exacerbation.

The pre-exacerbation period includes observations made at any time prior to the onset of the exacerbation. The beginning of the post-exacerbation period is determined with difficulty. It is believed to be present when clinical and laboratory findings have reached the pre-exacerbation level or have become stabilized.

Several instances of increased generalized edema in the absence of changes in the urinary findings were proved to be related to dietary deficiency and these cases were consequently not included in this study. In one such individual for example, a prolonged alcoholic bout with inadequate food intake resulted in massive edema. A hypoproteinemia well below the patient's previous serum protein level was present. The administration of an adequate diet resulted in a gain of 1 gram per 100 cc. in the serum albumin in 1 week with a coincident loss of edema. This phenomenon was apparently due to lack of dietary protein in a patient with nephritis and was not considered to be a true exacerbation.

Case material

For the past 8 years a large group of patients with chronic glomerulonephritis has been available for study in the Nephritis Hypertension clinic of Dr. Dana W. Atchley and Dr. Robert F. Loeb at the Presbyterian Hospital and a similar clinic of Dr. John D. Lyttle at the Babies Hospital. These patients have been closely followed in the clinic and have been hospitalized for appropriate studies. In special instances such patients have been under daily observation at the Research Service of the First Division of the Welfare Hospital. It is believed that the opportunity to investigate nephritis both in children and in adults yields a more complete picture of the natural course of

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disease than would be the case if the data were limited to findings in either children or adults

Of a total of 68 patients who have been studied for from 1 to 8 years, 13 have exhibited one or more exacerbations during our period of observation

Relation of age to exacerbation

Although adults make up the larger proportion of our series of patients with chronic glomerulonephritis, exacerbations have been detected chiefly in the children's group (Table I) The prepon-

TABLE I

Relation of age to exacerbations in chronic glomerulonephritis

Age groups*	Total number of cases of chronic glomerulonephritis	Total number of patients exhibiting exacerbations	Total number of exacerbations
14 or under	12	8	22
Over 14	57	5	6

* The age in each instance represents the age at death or the present age of those living

derance of exacerbations in the younger group does not appear to be related to the frequency of clinic or hospital visits Likewise, the extent of the study was similar for both groups Because of the higher rate for exacerbation in the younger group, the incidence of hospital admissions is greater for the children than for the adults

Incidence of exacerbation

Table II shows the incidence of the exacerbations in the 13 of our 68 patients with chronic glomerulonephritis where these episodes were observed Six of the patients had only one exacerbation during the period of study, whereas the remaining 7 developed from 2 to 5 exacerbations each A greater frequency of exacerbations per patient occurred in the younger age group

Relation of infection to the exacerbation in chronic glomerulonephritis

In each of the 28 exacerbations in this group, the patient was subjected to clinical, bacteriological and immunological study in order to determine the presence of prodromal infection This demonstration rested upon close clinical observation and repeated throat cultures for the determination of the presence of Group A hemolytic strepto-

coccus Serum antistreptolysin tests were carried out with a frequency sufficient to detect occult hemolytic streptococcus infections

Each of the 28 exacerbations followed infection (Table II) All of the 13 patients undergoing exacerbation of their disease had at least one exacerbation apparently related to a preceding hemolytic streptococcus infection (Table II) In all instances the hemolytic streptococcus infection was limited to the pharynx or adjacent tissues

Exacerbations in chronic glomerulonephritis following upper respiratory infection not proved due to the hemolytic streptococcus

Five of the 13 patients exhibited 8 exacerbations following upper respiratory infections in which the hemolytic streptococcus could not be proved the causative agent (Table II) More adequate study might have demonstrated the presence of hemolytic streptococcus infection in at least 3 of the 8 infections not proved due to hemolytic streptococcus Each of these patients on other occasions had an exacerbation apparently induced by hemolytic streptococcus infection

Latent period between prodromal infection and the onset of exacerbation

The latent period in each of the 28 instances between the onset of the prodromal infection and the onset of the exacerbation was as follows

Latent period	Number of exacerbations
1 day	12
2 days	5
3 days	2
4 days	2
7 days	1
14 days	1
Unknown	5

It is seen that, in the main, this latent period is a short one in contrast to that seen in acute glomerulonephritis The observations confirm those made by others (1, 2) It was found that the latent periods for the exacerbations following hemolytic streptococcus infection are approximately the same as those found following upper respiratory infections not proved due to the hemolytic streptococcus

The rapidity with which upper respiratory infection induces an increase in activity in chronic glomerulonephritis is of interest with regard to

TABLE II
Exacerbations in 13 patients with chronic glomerulonephritis

1		2	3	4	5	6	7	8	9	10
Name	Age	Total months of observation	Number of hospital admissions	Number of out-patient department visits	Exacerbations after hemolytic streptococcus infections	Exacerbations after upper respiratory infections not proved due to hemolytic streptococcus	Exacerbations unrelated to infection	Effect of exacerbations on renal function*		
								None demonstrated	Transient decrease	Permanent decrease
Ca	4	22	5	12	I II III	1	0	I II III 1		
Bl	9	37	8	16	I II III	1 2	0	1	I II III 2	
Ro	12	31	6	13	I II	1† 2† 3	0	1 2 3	I II	
Go	13	48	5	28	I		0		I	
Al	13	24	7	22	I II		0	I	II	
Gon	13	9	3	3	I II		0		I II	
Wh	14	40	5	6	I		0		I	
Le	14	16	1	9	I	1	0	I 1		
Ke	15	23	4	39	I		0		I	
Ed	17	6	3	5	I		0			I
Pa	17	6	2	4	I		0		I	
Du	18	55	5	20	I	1	0	I	1 (?)	
Mo	33	32	2	16	I		0		I	

* The Roman and Arabic numerals correspond to those used in columns 5 and 6

† May have been infection due to hemolytic streptococcus.

the concept that the kidney in this disease is hypersensitive to products of the hemolytic streptococcus. It has been shown (3) that as little as 10 skin test doses of a streptococcus hemolyticus nucleo-protein (4) may produce hematuria in less than 24 hours when injected intravenously into patients with chronic glomerulonephritis.

Effect of the exacerbation on renal function

In order to evaluate the effect of any single exacerbation on the renal function of the patient with chronic glomerulonephritis it is at once ap-

parent that observations during a prolonged fore period must be at hand as well as repeated studies in the post-exacerbation period. Since patients with chronic glomerulonephritis remain surprisingly well in the absence of gross renal failure it is difficult to interest some of these individuals in the discipline of clinical investigation unless there is an unusually cooperative attitude on the part of the patient. In the majority of instances it has been possible to obtain satisfactory data, but in some cases the failure of the patient to appear at frequent intervals for study

irreparable gaps in the natural history of the phenomena of exacerbation. For the most part, however, the data obtained appear valid from this point of view.

The status of the renal function was established chiefly by the urea clearance determination, the phenolsulphonphthalein excretion test, the urea ratio value of Mosenthal and Bruger (5), and the serum non-protein nitrogen level. During the periods between exacerbations of the nephritis some of these tests were performed at 1- to 3-month intervals. With the occurrence of an exacerbation, selected tests for renal function were usually conducted at weekly intervals. These tests were carried out under the conditions existing in routine hospital and clinic practice. The limitations and the difficulties in interpretation are obvious, but for the purpose of this study the tests are taken to indicate the relative rather than the absolute changes in renal function.

It is seen from Table II that 15 of the 28 exacerbations observed in this series of 13 patients produced a transient drop in renal function. Thirteen of these 15 exacerbations were apparently initiated by a Group A hemolytic streptococcus infection of the pharynx or adjacent tissues. The prodromal upper respiratory infection in the remaining 2 exacerbations was not proved due to the hemolytic streptococcus.

One exacerbation resulted in an apparent permanent decrease in renal function. A reading of the individual case history (Case IX) will indicate the difficulty experienced in evaluating the effect of the exacerbation in patients in the terminal stage of nephritis. It cannot be conclusively stated that the decrease in function, leading to death by uremia in this instance, was dependent upon the exacerbation.

Twelve of the 28 exacerbations failed to produce a demonstrable effect on renal function. These 12 exacerbations occurred in 6 patients, 4 of whom experienced other exacerbations in which a transient decrease of renal function was apparent. One of the remaining patients (Ca, Case I) showed no decrease in renal function during any of his 4 exacerbations, 3 of which followed Group A hemolytic streptococcus infection. The fourth exacerbation followed an upper respiratory infection not proved due to the hemolytic streptococcus. It is possible that our inability to demonstrate a

decrease in renal function following 12 of the exacerbations reported here is related either to the slight degree of the renal flare-up or to our failure to employ appropriate renal function tests at sufficiently frequent intervals.

DISCUSSION

The 13 patients² who experienced exacerbations of glomerulonephritis have been observed in the following manner. The tests for the activity of the nephritis were the usual ones. In addition to routine urinalyses, frequent Addis counts were performed. Records were made of the blood pressure, eyeground findings, serum protein values, the presence of edema, and the hemoglobin and red blood cell count values to determine the progress of the disease. The tests for renal function and the methods used for the demonstration of the presence or absence of hemolytic streptococcus infection have been described above.

Although there is agreement that hemolytic streptococcus infection is the chief initiating factor in acute glomerulonephritis, doubt prevails concerning the agent responsible for the maintenance and progression of the disease *chronic glomerulonephritis*. The failure to reproduce chronic glomerulonephritis experimentally in animals with the hemolytic streptococcus limits the investigation of the role of infection in this disease to clinical studies. The major work in this field has been carried on by Longcope and his associates (6, 7, 8). Winkenwerder, McLeod and Baker (2) analyzed the data accumulated by Longcope and concluded that "Persistence of the streptococcus was characteristic of the chronic stage of the disease." These workers report the contributions of others whose opinions are pertinent to the question.

In the past 8 years at the Presbyterian and Babies Hospitals in New York City it has been possible to collect evidence concerning the relation between infection, particularly of the upper respiratory tract, and the course of chronic glomerulonephritis in 68 patients. A report of the degree of parallelism which exists between the presence of infection and the velocity of the nephritis in this group of patients is now being prepared. The present communication is limited to the study of

² See protocols

the exacerbations in chronic glomerulonephritis. The chief points to be determined were (1) the frequency with which exacerbation occurs, (2) the infectious factors initiating the exacerbation, and (3) the effect of the latter upon the course of the nephritis

The data which have been presented indicate that in this series of 68 patients observed from 1 to 8 years unequivocal exacerbation occurred in only 13 members of the group. Attention has been directed to the difficulty of delimiting the criteria necessary to determine the presence of the exacerbation. A sharp increase in the degree of hematuria occurred in 4 patients who have not been included in this series. The case histories of the individuals all adults have been excluded for the following reasons

Patient Ar. The hematuria which occurred under our observation followed the intravenous injection of a hemolytic streptococcus nucleoproten.

Patient Pf. The hematuria was always so variable it was impossible to describe any single episode as indicative of an exacerbation

Patient Fo. The hematuria occurred terminally and the patient was not available for adequate study

Patient Hu. This patient was observed early in our study and adequate immunological and renal function tests were not performed.

The addition of these cases to our series would not have altered the conclusions beyond increasing the number of individuals in the adult group

In our experience frank exacerbation in chronic glomerulonephritis occurs only after infection. As a rule, this infection is caused by Group A hemolytic streptococcus. Winkenwerder, McLeod and Baker (2) studied the etiological factors in 42 exacerbations in 'chronic hemorrhagic nephritis.' They found that 42 of 126 infections which occurred during the course of the disease in their series appeared to initiate an exacerbation. Fifty five per cent of these infections were shown to be of hemolytic streptococcus origin. They further observed that 30 per cent of 84 infections unaccompanied by exacerbation were also of hemolytic streptococcus origin. These workers believed that their data indicated that when infection is followed by exacerbation of nephritis streptococcus is apt to be found"

In our series of 68 patients with chronic glomerulonephritis there were 350 infections of various types, of which 68 were shown to be of hemolytic streptococcus origin. Twenty (71 per cent) of the 28 exacerbations observed in the entire group followed hemolytic streptococcus infection whereas only 48 hemolytic streptococcus infections occurred among the 322 other infections (15 per cent) which failed to incite an appreciable exacerbation. In this respect, our data confirm those presented by Winkenwerder and his associates. The lower percentage of hemolytic streptococcus disease among infections failing to produce exacerbations in our group may be due to the greater frequency of visits of patients. Because of this the number of mild infections (not of hemolytic streptococcus origin) of the upper respiratory tract which we have observed may be greater than that observed by Winkenwerder, McLeod and Baker. It should be emphasized that all infections occurring during the acute and early subacute stages of glomerulonephritis are excluded from this survey

As already stated careful study of our patients during the period of exacerbations shows that each of these episodes was preceded by an infection. Winkenwerder, McLeod and Baker, however, have observed exacerbations of nephritis "despite the failure to demonstrate any inciting factor." This divergence may depend upon the interpretation of the criteria required to define exacerbation. It may also rest in part upon the fact that clinical and bacteriological determination of the presence of hemolytic streptococcus infection may fail in some cases to yield positive data. With the use of the antistreptolysin test, however, the presence of hemolytic streptococcus infection may be detected in the absence of bacteriological data. It is possible that some of the exacerbations in Winkenwerder, McLeod and Baker's series were observed prior to the general use of this test

Our accumulated evidence demonstrates that a transient decrease in renal function is a frequent result of the exacerbation in chronic glomerulonephritis. Fifteen of the exacerbations effected such a temporary decrease in renal function. In contrast 12 exacerbations could not be shown to have altered the functional level of the kidneys. The exacerbations in this latter group were in

initiated by hemolytic streptococcus infection in 6 instances and the remaining exacerbations followed upper respiratory infections in which the hemolytic streptococcus could not be proved the causative agent. A permanent and apparently progressive decrease in renal function followed an exacerbation in only one patient in our series. In this instance the terminal state of nephritis was at hand when the exacerbation appeared. It is obviously difficult to evaluate significant changes in renal function in patients who are in the terminal stage of chronic glomerulonephritis.

The failure of the majority of exacerbations in this series to produce a permanent decrease in renal function was surprising to us. It was felt that if repeated or continuous hemolytic streptococcus infection was the mechanism which caused progressive renal damage in patients with chronic glomerulonephritis, one would expect that the hemolytic streptococcus infection which induced an exacerbation of nephritis would be particularly effective in permanently reducing renal reserve. The analysis of the 27 exacerbations reported here lends little evidence to this concept unless the suggestion is accepted that a single hemolytic streptococcus infection in a patient with chronic glomerulonephritis can initiate a type of renal damage which perpetuates itself in autocatalytic form for months or years.

It is possible that the return of the functional values to the pre-exacerbation level after the depression induced by the renal flare-up is more apparent than real. Although the post-exacerbation tests for renal function may be normal, nevertheless a reduction of the mass of functioning renal tissue may have occurred. This possibility is shown diagrammatically in Figure 10 (A).

In this hypothetical instance 3 exacerbations of nephritis have induced 2 periods of "transient" and 1 period of "permanent" decrease in renal function. However, a reduction in the per cent of functioning renal tissue has occurred following the first 2 exacerbations despite our inability to demonstrate this state by the usual function tests. This difficulty is partially met in instances where exacerbation occurs in an individual with chronic glomerulonephritis whose renal function levels are already decreased.

An alternative hypothesis to that shown in Fig-

ure 10 (A) is diagrammatically shown in Figure 10 (B). This mechanism presumes that the velocity of the nephritis is established at its initiation through an unknown cause. Exacerbations produce dramatic episodes but do not significantly alter the slope of the curve. It is impossible at this time to designate either hypothesis as correct.

Addis (9) in 1931 stated that in his experience none of the infections and incidents which led to temporary increases in the intensity of the renal lesion seemed to have resulted in any irreparable loss of secreting tissue. He further described 2 cases in which repeated exacerbations seemed not to be accompanied by a significant decrease in the "amount of functioning renal tissue." Winklerwerder, McLeod and Baker (2) have shown "that recurring infections, in spite of their relation to exacerbations, do not necessarily determine the ultimate outcome of the disease, for partial recovery to the latent stage or complete recovery from nephritis was not prevented by such infection, and whereas the progressive group was characterized by recurring infections in some cases, in others this factor was not obvious and in a few patients active infection was never demonstrated." Osman (10), who has studied the "late-results of 'acute nephritis,'" believes that the number of attacks of nephritis (exacerbations?) has apparently no influence on the ultimate prognosis. Leiter (11) has stated that in most patients with chronic nephritis "further decrease of function occurs as the result of definite acute exacerbations following acute streptococcal infections as the result of slow but continued destruction of renal parenchyma in the absence of obvious infection anywhere in the body or because of organic vascular changes in the kidney."

It seems evident, therefore, that the exacerbation in chronic glomerulonephritis is initiated chiefly by hemolytic streptococcus infection and that the exacerbation usually effects only a transient drop in renal function.

Exacerbation as defined here is seen to be chiefly a manifestation of childhood glomerulonephritis. Although the adult group has been subjected to careful study, the incidence of exacerbation as defined here has been low. There is only one patient in this group exhibiting exacerbation over the age of 20.

SUMMARY AND CONCLUSIONS

1 The natural history of each of 28 exacerbations of nephritis occurring in 13 of a series of 68 patients with chronic glomerulonephritis is described. In the main, these individuals were under close observation for periods of from 1 to 8 years.

2 An abrupt, marked increase in hematuria with or without a concomitant impairment of renal function is assumed to indicate the occurrence of an exacerbation in chronic glomerulonephritis.

3 The frequency of exacerbation so defined is greater in the young than in the adult group in this series.

4 All 13 of the patients experienced exacerbations initiated by Group A hemolytic streptococcus infection. Five of these patients underwent additional exacerbations related to upper respiratory infections which could not be proved due to the Group A hemolytic streptococcus.

5 In the total series of 68 patients with chronic glomerulonephritis there were 350 infections of various types of which 68 were shown to be of hemolytic streptococcus origin. Twenty (71 per cent) of the 28 exacerbations followed hemolytic streptococcus infection whereas only 48 hemolytic streptococcus infections occurred among the 322 (15 per cent) infections which failed to incite an appreciable exacerbation.

6 No exacerbation of chronic glomerulonephritis in this series occurred without concomitant infection.

7 The latent period between the onset of the infection and the exacerbation was from 1 to 4 days in the majority of instances. This latent period as pointed out by others is much shorter than that seen between the infection and the onset of acute glomerulonephritis.

8 A common effect of the exacerbation was to produce a transient decrease in renal function. Ten of the 13 patients exhibited 1 to 4 such exacerbations.

Six patients underwent exacerbations in which no effect on renal function could be demonstrated. Four of these 6 patients however experienced other exacerbations in which a transient decrease in renal function occurred. Only 1 patient developed a permanent decrease in renal function

following an exacerbation but the nephritis at this time was in the terminal stage.

9 It may be concluded that the exacerbation in chronic glomerulonephritis is the result of infection and that this infection is due mainly to invasion by the Group A hemolytic streptococcus. The chief effect of the exacerbation is the production of a transient rather than a permanent decrease in renal function. However since there is no correlation between mass of functioning renal tissue and renal function tests when the latter are within the range of normal, it is impossible to determine whether or not the total mass of renal tissue has been reduced in those cases where renal function tests indicate only a transient decrease in function.

Dr David P. Earle, Jr., assisted in the collection of the data from several of the patients in this series.

PROTOCOLS

Case 1 (Ca)

This 4-year old boy was first seen by us on January 26, 1938, in what appeared to be a typical attack of acute glomerulonephritis. However since there was an equivocal record of hematuria associated with fever 6 months earlier it was believed that the nephritis probably was initiated at that time.

Certain details of the episodes of exacerbation of the nephritis on January 18, 1938, March 20, 1938, January 5, 1939 and April 26, 1939 are shown in Figure 1. It is seen here that data are available on the degree of albuminuria, hematuria, edema, blood pressure, nonprotein nitrogen of the serum and the urea ratio prior to, during, and after the respective exacerbations of the nephritis.

It is apparent that the patient experienced 4 exacerbations of nephritis over a 15-month period. In each exacerbation of his disease there was an increase in albuminuria and hematuria above the previous base line, but it is also evident that in each instance these urinary findings returned to the pre-exacerbation level. On the basis of the other evidence at hand with respect to edema, blood pressure, serum nonprotein nitrogen and urea ratio determinations it cannot be shown that a significant drop in renal function is associated with any of these exacerbations. A single urea clearance test performed during the third exacerbation showed a value of 97 per cent of normal (not shown on chart).

The findings in this patient with respect to the effect of exacerbation on renal function differ from those seen in the majority of our cases in that no significant depression in renal function is associated with the exacerbations. It is possible, however that frequent urea clearance determinations and additional renal function tests might have detected such a change.

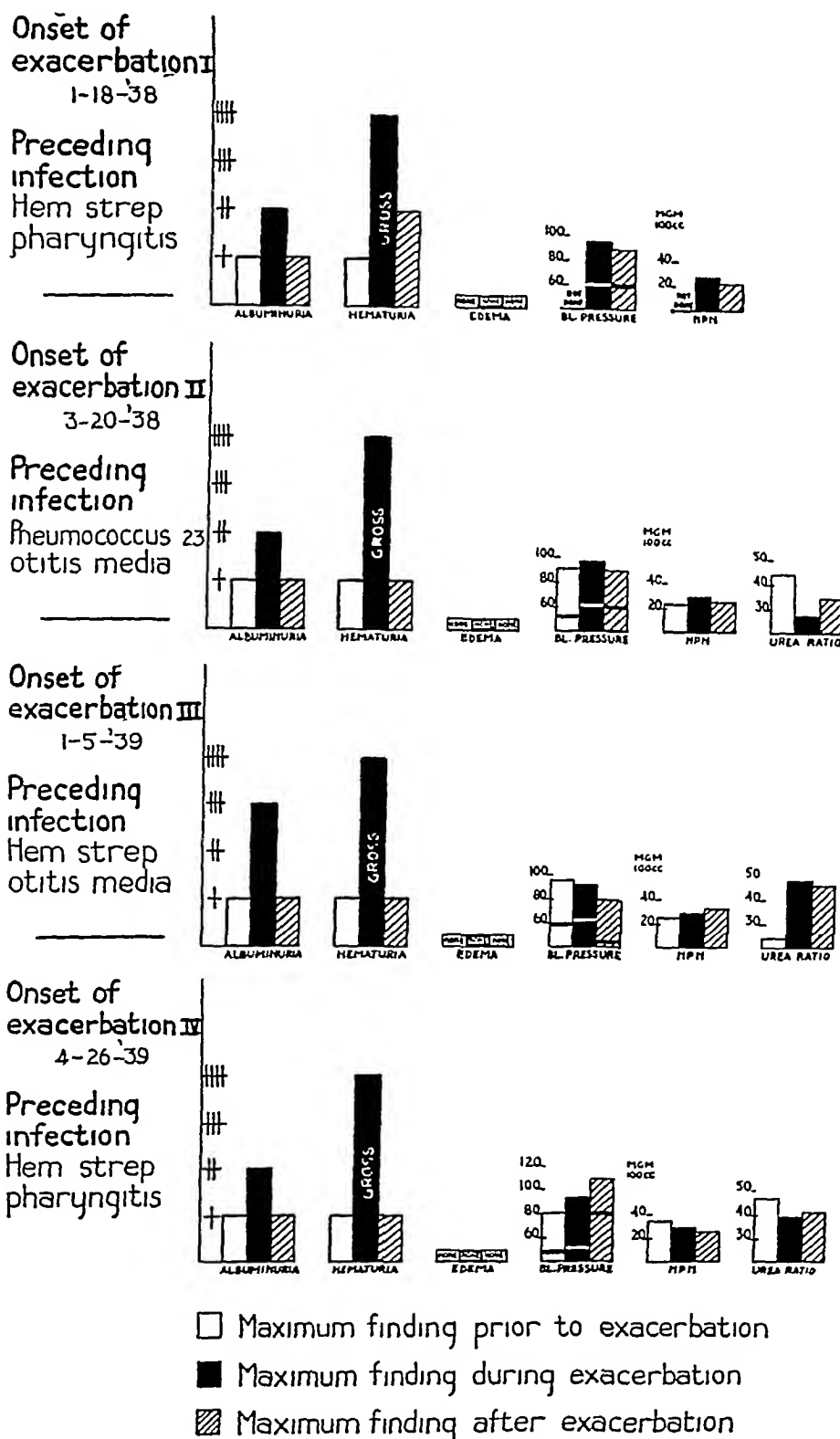


FIG. 1 CASE I (Ca) OBSERVATIONS BEFORE, DURING, AND AFTER FOUR EXACERBATIONS IN COURSE OF CHRONIC GLOMERULONEPHRITIS

Case II (BI)

This boy who is now 9 years old, was said to have had "kidney trouble" following scarlet fever at the age of 4 (1934). In 1935 there was a history suggesting an exacerbation of the nephritis occurring during an episode of mastoiditis for which a mastoidectomy was performed.

He was first seen by us in May 1936 when an incision and drainage was performed for the relief of inguinal lymphadenitis with abscess. The culture of the pus yielded "hemolytic staphylococcus aureus" but the anti streptolysin level was found to be 250 units. Four months later this value had dropped to 111 units. There was no evidence for an exacerbation of the nephritis associated with this infection.

During the next 37 months of observation in which there were 8 hospital admissions and 16 clinic visits, there were 5 exacerbations 4 of which were associated with a transient decrease in renal function.

Since all these exacerbations occurred between July, 1938 and April 1939 it was impossible to determine accurately the fore period for each bout. The natural history of the nephritis and associated infections is herewith described.

Exacerbation I occurred on July 3 1938, following an *H influenzae* otitis media. There was no evidence for hemolytic streptococcus infection at this time. The hematuria, which was minimal (\pm) before the flare-up became ++++ during the exacerbation. A +++ albuminuria became ++++ during the renal flare-up and remained at this level permanently. There was no evidence for an associated depression in renal function. The urea clearance of 60 per cent of normal and the

urea ratio of 50 remained constant throughout the exacerbation and thereafter. Following this acute episode the hematuria returned to its previous level.

Exacerbation II occurred on August 25, 1938. This was apparently related to a hemolytic streptococcus pharyngitis. *S. hemolyticus* and *H influenzae* were cultivated from the pharynx, but evidence for invasion by hemolytic streptococci was shown by the rise of the anti streptolysin titer from 62 to 166 units. Hematuria edema and the serum nonprotein nitrogen level increased through this bout only to return to the previous approximate base line in the post-exacerbation period. These data are shown below

	Before exacerbation	During exacerbation	After exacerbation
Hematuria	+	+++	+
Edema	\pm	++	\pm
Nonprotein nitrogen	25	46	33

Exacerbation III occurred on October 14 1938. This was apparently related to a mild pharyngitis. Group A hemolytic streptococci were isolated from the throat culture and the antistreptolysin titer rose from a base line of 83 units to a peak of 166 units. The hematuria, which was minimal (\pm) before the exacerbation, became ++++ during the exacerbation and dropped to + with the termination of the bout. Although there were no significant changes in edema, blood pressure, and the serum nonprotein nitrogen values and urea clearance figures during this episode, the base line urea ratio of 40

Explanation of Figures on Chart

The values for the albuminuria are the conventional ones.

In this and subsequent charts the hematuria, as determined in the sediment of the usual centrifuged specimen, is designated in the following manner

1-5 red blood cells per H.P.F	+
6-15 red blood cells per H.P.F	++
16-25 red blood cells per H.P.F	+++
Above 25 red blood cells per H.P.F	++++

The presence of gross hematuria is so indicated.

In a number of the patients Addis counts were made to determine the degree of hematuria. This finding is so designated on the graph.

The values for edema are expressed as follows

Slightest demonstrable edema	+
Edema of face and ankles	++
Generalized edema with fluid in visceral cavity	+++

The figure +++ is arbitrarily used to describe the degree of edema between ++ and ++++

The values for the systolic blood pressure level are designated by the height of the column. The heavy horizontal bar shows the diastolic peak.

The nonprotein nitrogen levels are expressed as serum values. The figures for the urea ratio urea clearance and phenolsulphonphthalein excretion tests are self-explanatory

rose to 70 and dropped to 55 after the exacerbation. Possibly too much reliance should not be placed on the urea ratio values here since no comparable changes occurred in the urea clearance figures

Exacerbation IV occurred on March 7, 1939. This episode was preceded by abdominal pain, vomiting, temperature of 102°, and isolation from the blood of a non-typeable pneumococcus. From clinical evidence it was felt that a pneumococcus peritonitis existed, but this point was not proved. Sulfapyridine therapy cured the pneumococcus infection. Antistreptolysin determinations showed values within normal limits. This infection apparently induced a transient decrease in renal function as is shown below

	Before exacerbation	During exacerbation	After exacerbation
Hematuria	+	+++	+
Edema	+	+++	+
Nonprotein nitrogen	31	58	28
Urea clearance	40%	38%	60%
Urea ratio	55	68	38

Exacerbation V, which occurred on April 1, 1939, followed an otitis media with post-auricular abscess. Group A hemolytic streptococci were isolated from the abscess exudate and the antistreptolysin titer rose from a base line of 25 to 33 units to a peak of 715 units. This exacerbation was characterized by the following changes in the renal status

	Before exacerbation	During exacerbation	After exacerbation
Hematuria	+	++	±
Edema	±	+	±
Blood pressure	140/90	160/100	130/90
Urea clearance	38%	38%	60%
Nonprotein nitrogen	32	43	27
Urea ratio		61	38

It is probable that a significant transient decrease in renal function occurred during this exacerbation.

The course of this patient's nephritis during 5 exacerbations of the disease is of interest with respect to the comparative functional level before and after the renal flare-ups. A transient decrease in function was demonstrated during the 3 exacerbations related to hemolytic streptococcus infection and the single exacerbation apparently due to the non-typeable pneumococcus infection. No change in renal function was observed during the exacerbation which apparently followed the *H influenzae* otitis media. It is further seen that a comparison of the renal competence before and after the 5 exacerbations which occurred between July 1938 and April, 1939 shows no striking difference. The urea clearance on June 22, 1938, was 63 per cent of normal, on June 2, 1939, 60. The urea ratio on June 22, 1938, was 43, on June 2, 1939, 38 (normal below 50). The serum non-protein nitrogen value was 33 mgm. per 100 cc. on June

22, 1938, and 27 mgm. per 100 cc. on June 2, 1939. It is evident, therefore, that in this patient frequent exacerbations of glomerulonephritis have resulted in only transient drops in renal function.

Case III (Ro)

This girl, who is now 12 years old, has one sibling who has had both rheumatic fever and glomerulonephritis. The onset of acute nephritis in our patient probably occurred in July, 1932. From the history it would appear that exacerbations of the nephritis occurred in December, 1932, after a bronchitis, in May, 1936, after a sore throat, and in August, 1936, from an unknown cause.

The patient first came under close observation by us in September, 1936. During the subsequent 3 years she was studied through the course of 5 exacerbations of nephritis. An additional exacerbation occurred outside our observation. This bout is not included in our description. It is seen from Figure 2 that all 5 exacerbations were characterized by a sharp increase in the degree of albuminuria and hematuria. In all instances there was a decrease in this abnormality to the approximate basal level after the renal flare-up.

Exacerbation I occurred on March 12, 1937, when the patient was 10 years of age; it appeared to be related to a preceding infection described as "grippe." The available data cannot exclude the possibility that the hemolytic streptococcus played a role in this infection. The evidence at hand suggests that this exacerbation failed to produce a drop in renal function.

Exacerbation II occurred on May 26, 1937, and was preceded by a hemolytic streptococcus pharyngitis. The antistreptolysin titer rose from a base line of 144 units to 715 units. This exacerbation induced a temporary drop in renal function. There was a transient + edema and the serum nonprotein nitrogen level rose from 22 to 59 and then dropped to 28 mgm. per 100 cc in the post-exacerbation period.

Exacerbation III occurred on October 28, 1937, and was preceded by lobar pneumonia. Pneumococci could not be isolated from the sputum. During a relapse of the pneumonia 1 month later, in which gross hematuria reappeared, hemolytic streptococci and not pneumococci were isolated from the sputum. Antistreptolysin determinations were not carried out at sufficiently frequent intervals to rule out the possibility that the pneumonia was of hemolytic streptococcus origin. There was no evidence of disturbance in renal function during this flare-up.

Exacerbation IV occurred on May 1, 1938, and was preceded by a hemolytic streptococcus pharyngitis. The throat cultures during this episode showed the predominant organism to be a Group A hemolytic streptococcus. The base line antistreptolysin titer of 333 units remained unchanged following the sore throat. Nevertheless, it was felt that infection by the hemolytic streptococcus had occurred. During this exacerbation (Figure 2) a transient edema, hypertension, slight rise in the serum non-protein nitrogen value and a sharp rise in the urea ratio developed. The urea clearance during this bout was

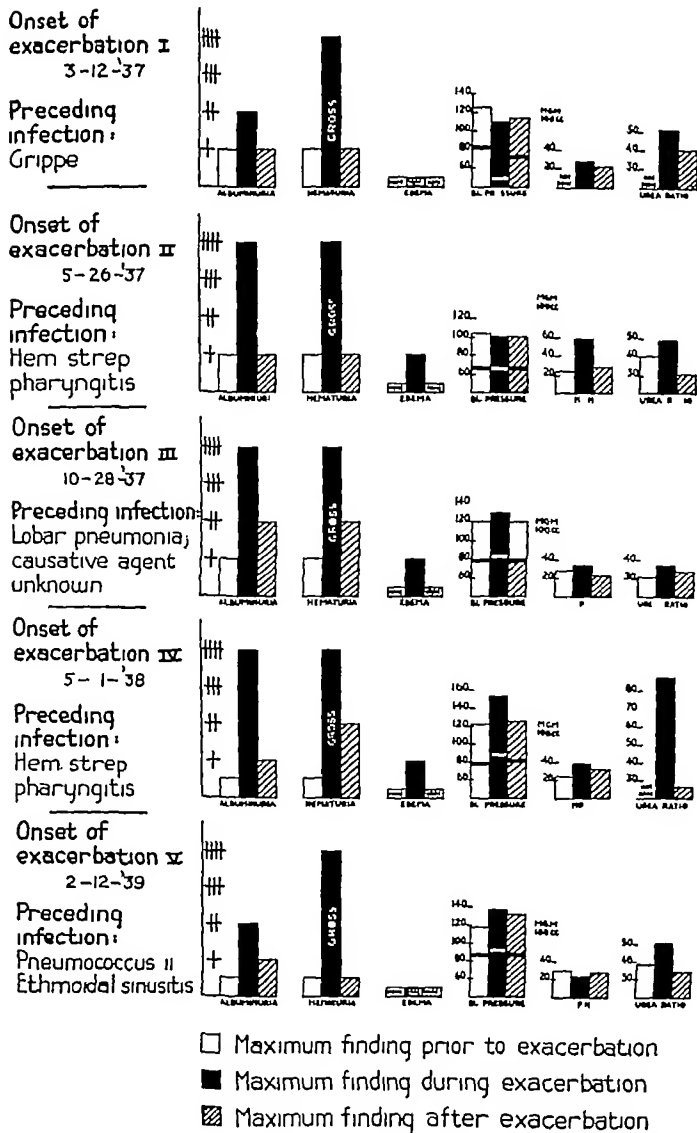


FIG. 2. CASE III (Ro) OBSERVATIONS BEFORE, DURING, AND AFTER FIVE EXACERBATIONS IN COURSE OF CHRONIC GLOMERULONEPHRITIS

found to be 28 per cent and 29 per cent of normal on 2 occasions. Nine months later the urea clearance was 76 per cent of normal.

Exacerbation V developed on February 12, 1939, and was preceded by an ethmoidal sinusitis. Cultures of the pharyngeal exudate showed pneumococcus type XI as the predominating organism. There was no evidence to implicate the hemolytic streptococcus in this infection. The exacerbation in this instance failed to produce any demonstrable effect on renal function.

In summary, this 12-year-old girl with chronic glomerulonephritis of 7 years' duration has been studied through 5 exacerbations in the past 3 years. The 2 exacerbations apparently related to hemolytic streptococcus infection caused a temporary drop in renal function. The exacerbation said to be related to pneumonia is shown to have resulted only in a transient edema. The remaining 2 exacerbations failed to effect detectable drops in renal function. It is of interest that the urea clearance of 28 per cent of normal found during the fourth exacerbation on May 1, 1938, had risen to 76 per cent of normal during the fifth exacerbation on February 15, 1939.

Despite the 5 exacerbations which were observed by us and 4 other exacerbations deduced from the history, this patient shows remarkably good functional reserve at the end of 7 years of extremely active glomerulonephritis, if the frequency of exacerbation is accepted as a criterion of activity. Not only is the urea clearance 76 per cent of normal but the serum nonprotein nitrogen level is 22 mgm. per 100 cc. and the urea ratio is 45. The blood pressure is 126/82, there is no edema, the

albuminuria is only + and there are so few red blood cells in the urine that the benzidine test is negative.

Case IV (Go)

This boy died of uremia in March, 1939, at the age of 13. In 1931 at the age of 5 he had had scarlet fever and otitis media. Adequate urinalyses were not performed at that time. Four years later, in March, 1935, he had a severe sore throat followed in 2 weeks by otitis media and edema of the face and ankles. On admission to the Babies Hospital in April, 1935, he was found to have marked anemia, moderate generalized edema, hypertension (176/130), slight vascular changes in the ocular fundi, gross hematuria, heavy albuminuria, and numerous granular, hyalin, and red blood cell casts in the urinary sediment. The nonprotein nitrogen level was 55 mgm per 100 cc. and the maximum specific gravity of the urine was 1.012. The clinical opinion was that the picture represented an exacerbation in chronic glomerulonephritis, rather than an initial attack of acute glomerulonephritis. It was believed that the latter may have resulted from the scarlet fever in 1931. Despite the presence of chronic pharyngitis, hemolytic streptococci could not be isolated by culture. The antistreptolysin titer, however, was 144 units. This value subsequently dropped to 71 units. This is suggestive evidence that the pharyngitis and otitis media were probably due to the hemolytic streptococcus.

From Figure 3 it is seen that this exacerbation resulted in edema, hypertension and nitrogen retention. There was a reduction in these three abnormalities after the exacerbation.

The subsequent history of this patient shows a gradual

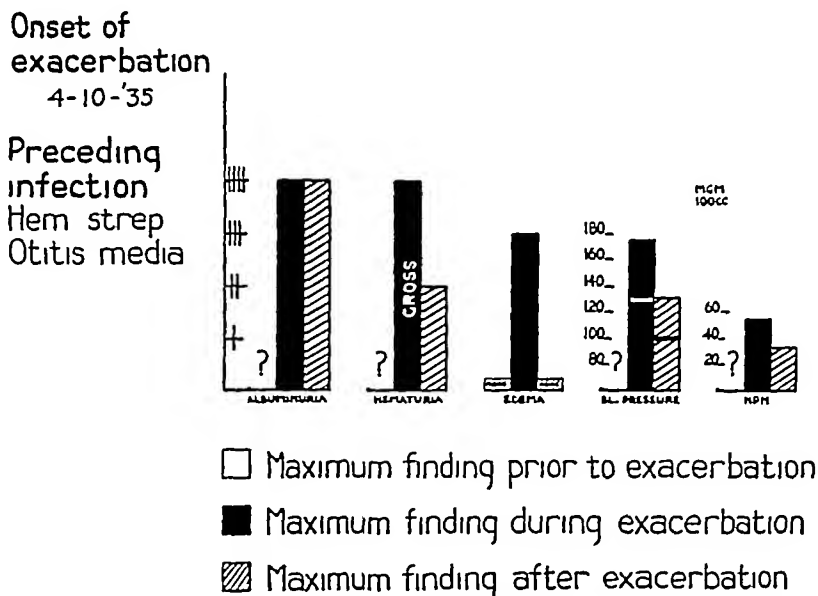


FIG. 3 CASE IV (Go) OBSERVATIONS BEFORE, DURING, AND AFTER EXACERBATION IN COURSE OF CHRONIC GLOMERULONEPHRITIS

reduction in renal competence until death 4 years later in March, 1939

Case V (Al)

This girl died in December 1937 of uremia and cardiac failure at the age of 13. Three years earlier she had developed her first symptoms suggesting nephritis, when hospital examination showed the presence of marked albuminuria, moderate hematuria, moderate generalized edema, cardiac hypertrophy and hypertension (148/100)

The serum nonprotein nitrogen was 50 mgm. per 100 cc., she was unable to concentrate the urine above 1.015 and the urea clearance was 36 per cent of normal. Chronically diseased tonsils were removed without incident. On discharge from the hospital the hypertension, edema and urinary findings persisted. Her urea clearance had now dropped to 12 per cent of normal and the urine concentration test was fixed at 1010

The patient first came under our observation in December 1935 2 years before her death. At this time, in addition to the manifestations of chronic glomerulonephritis, a marked pansinusitis was found. The exudate from available sinus orifices repeatedly yielded Group A hemolytic streptococci on culture. A bilateral maxillary sinusotomy was performed on December 31 1935 and again on March 3 1936 without apparent modification of the nephritic process.

Exacerbation I occurred on February 7 1936, within 24 hours following the development of an upper respiratory infection and a flare-up of the sinusitis. Within 2 weeks an otitis media developed, but this complication subsided without operation. Group A hemolytic streptococci were isolated from the pharynx during the upper respiratory infection and within 6 weeks the antistreptolysin titer had risen from the base line of 55 units to a peak of 333 units. From Figure 4 it is observed that no striking changes occurred in the edema level, the blood pressure values or the nonprotein nitrogen figure during this exacerbation

Exacerbation II occurred on February 5 1937 and was preceded by a sore throat 4 days earlier. Group A hemolytic streptococci were isolated from the pharynx and the antistreptolysin titer rose from a base line of 71 units to 166 units within 2 weeks. From the chart it may be seen that there was a sharp drop in kidney function associated with this renal flare-up. The serum nonprotein nitrogen level showed a rise from 55 to 95 mgm. per 100 cc. and the urea ratio reached a high level of 82. Following the exacerbation the nonprotein nitrogen returned to its approximate pre-exacerbation level and there was striking improvement in the urea ratio.

The subsequent history of this patient was one of progressive decrease in renal reserve, with hypertension and cerebral hemorrhage dominating the picture. There was almost continuous hemolytic streptococcus infection during the 2 year period in which we observed her. Although the presence of this infection may have contributed to the progressive renal damage, it may be stated

that when opportunity was available to follow the patient through a typical bout of hemolytic streptococcus invasion, the harmful effect of renal function was transient. It cannot be denied, however that the persistence of the hemolytic streptococcus sinusitis may have contributed to progressive renal damage in this patient.

Case VI (Gon)

This 13-year-old girl developed acute glomerulonephritis probably in April, 1936, following a hemolytic streptococcus cervical lymphadenitis with abscess formation. From her history it was learned that in July 1936 she had experienced a second attack of cervical lymphadenitis for which incision and drainage was performed. The hemolytic streptococcus was cultivated from the exudate. Slight abnormalities were observed in the urine during this episode but accurate data concerning these changes could not be obtained. The patient spent the next year in Southern Florida and was in apparent excellent health. There were no urinalyses during this period.

She was first seen at Babies Hospital on April 27 1938, with an exacerbation of nephritis which is described in Figure 5. On April 6 1938, there was swelling of the neck, vomiting and fever. One week later gross hematuria was observed. One week before admission, on April 20 1938, an incision and drainage was performed for the relief of the cervical abscess. When first seen by us on April 27 1938, the patient presented the typical picture of active glomerulonephritis. Cultures of the cervical abscess discharge yielded Group A hemolytic streptococcus. The antistreptolysin titer reached a peak of 715 units.

From the chart it is seen that the first exacerbation is characterized by an increase in albuminuria and hematuria. The figures for blood pressure, serum nonprotein nitrogen urea ratio and urea clearance show that the function had been impaired during the exacerbation and that improvement in renal reserve followed the flare-up. Since there is no adequate force period study with respect to the functional tests, it is impossible to compare the post-exacerbation with the pre-exacerbation levels.

Exacerbation II occurred on December 6 1938, and appeared to be associated with a recrudescence of the chronic cervical lymphadenitis. This lesion was drained a month later when Group A hemolytic streptococci were again obtained on culture of the purulent discharge. The antistreptolysin titer rose from a base line of 55 units to a peak of 250 units. From the chart it is seen that this exacerbation provoked a slight transient disturbance in kidney function, as shown by the increase in the urea ratio.

In summary this 13-year-old girl experienced 2 exacerbations of chronic glomerulonephritis which in each instance effected a transient decrease in renal function.

Case VII (Wh)

On August 4 1934 at the age of 10 this girl developed edema after a chill which was said

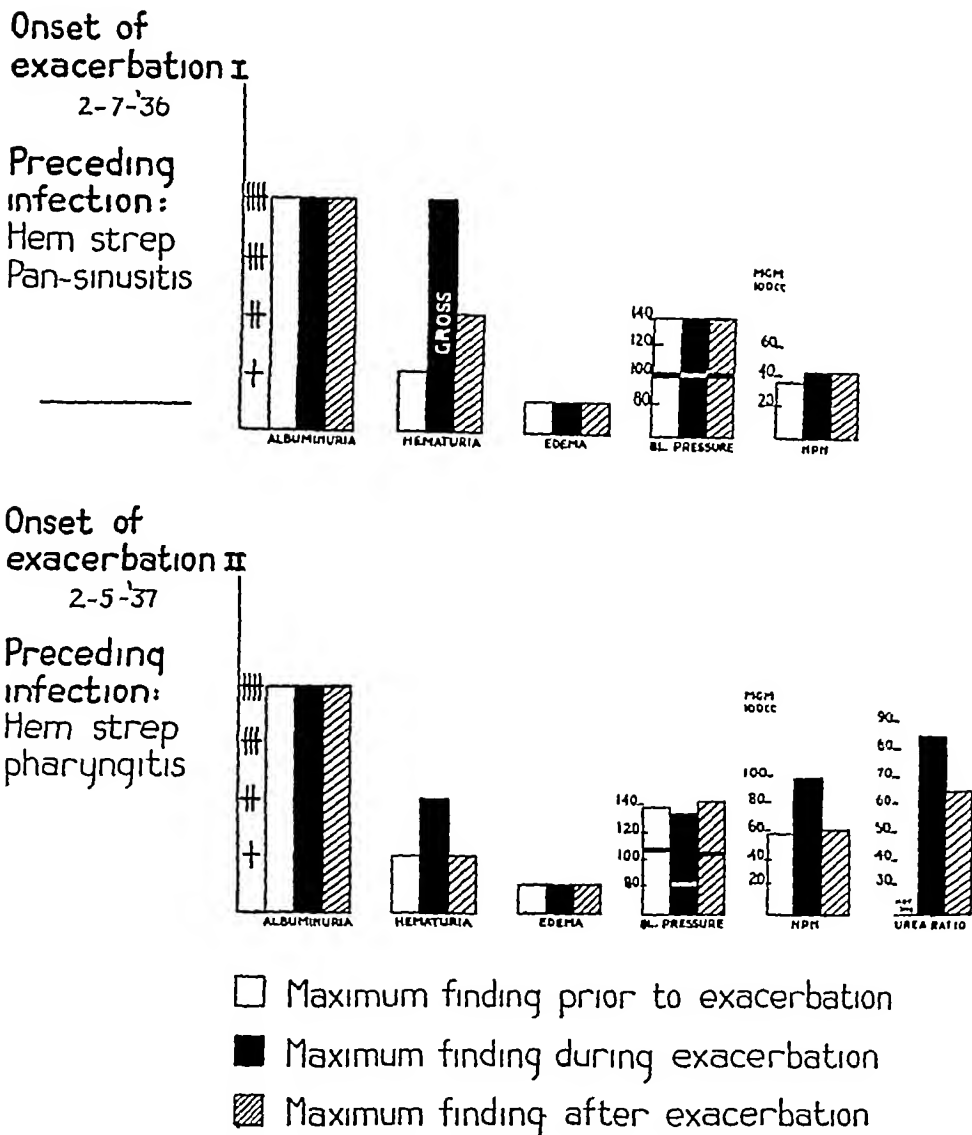


FIG. 4 CASE V (A1) OBSERVATIONS BEFORE, DURING, AND AFTER TWO EXACERBATIONS IN COURSE OF CHRONIC GLOMERULONEPHRITIS

induced by swimming. Heavy albuminuria and microscopic hematuria were reported but the blood pressure was not taken. She was first seen by us 5 weeks later on September 17, 1934. At this time she was found to have smoky urine, moderate edema of the face, sacrum and legs, a blood pressure of 190/160, and a serum non-protein nitrogen value of 44 mgm. per 100 cc. There was moderate cardiac enlargement and the fundi showed slight edema of the discs and arteriolar spasm. The serum albumin was 2.5 per cent and the serum cholesterol 475 mgm. per 100 cc. As far as one could tell, this episode represented the initial attack of nephritis.

There was no history of an acute infection prior to this renal episode beyond the description of the chill. However, there was evidence on admission of a chronic

pharyngitis and chronic tonsillitis. Group A hemolytic streptococci were cultivated from the throat exudate on admission and from the excised tonsils 3 months later. The antistreptolysin titer on admission was 33 units and remained at this level for the next 9 months.

On December 12, 1934, a tonsillectomy and adenoidectomy were performed. There were no modifications in the urinary findings, the blood pressure values, or degree of edema following this operative procedure. However, the urinary output was diminished for 2 days following operation and the serum nonprotein nitrogen rose from 46 to 50 but 4 days later it had decreased to 31 mgm. per 100 cc.

On February 7, 1935, she was admitted to the Babies Hospital for study. Although interval observations had

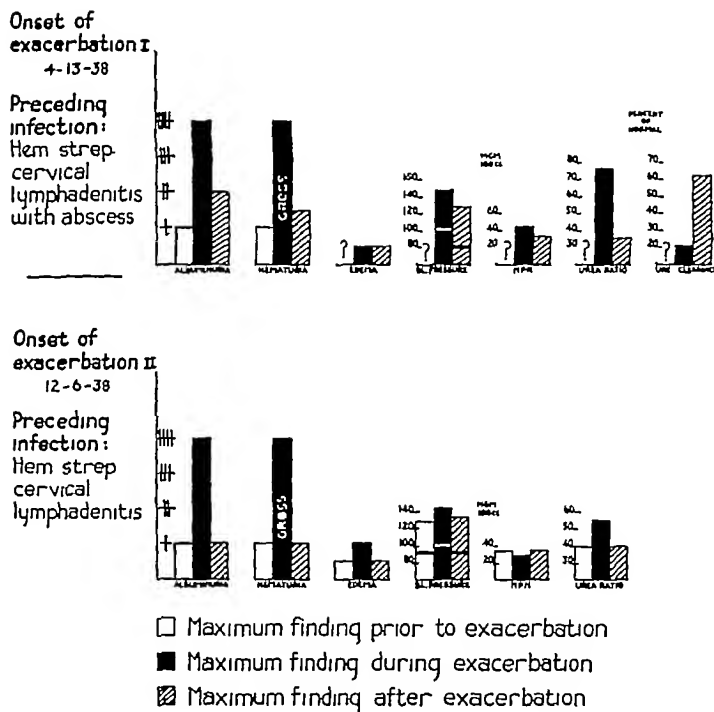


FIG 5. CASE VI (Gon) OBSERVATIONS BEFORE, DURING, AND AFTER TWO EXACERBATIONS IN COURSE OF CHRONIC GLOMERULONEPHRITIS

not been frequent, there appeared to be clinical improvement. With modified restriction of activity there were no symptoms referable to hypertension or cardiac strain. Facial edema was noted only occasionally and the urinary output was adequate. In the hospital gross hematuria and heavy albuminuria persisted. Slight edema of the face and ankles was present. The blood pressure was 180/130 and the serum nonprotein nitrogen was 36 mgm. per 100 cc. These findings were similar to those noted on discharge in December 1934.

On February 21 1935, this patient contracted scarlet fever. Group A hemolytic streptococci were isolated from the pharynx and the antistreptolysin titer rose from 25 to 333 units. Two and a half months later this value had dropped to 71 units. This antistreptolysin response was of interest in view of the low titer observed in August, 1934 despite the presence at that time of Group A hemolytic streptococci in the pharynx. It is probable that the patient was a carrier of the organism when first seen and that the hemolytic streptococcus had not invaded

the tissues sufficiently to produce an antistreptolysin response.

An exacerbation of the nephritis immediately followed the initial signs of scarlet fever. Since the changes in the urine were so marked prior to the onset of scarlet fever it was difficult to evaluate changes in this respect, but it was observed that the urine became red at the onset of the rash (Figure 6). Although there were no significant changes in the degree of edema or blood pressure, the serum nonprotein nitrogen level rose from 36 to 86 mgm. per 100 cc. during the course of the scarlet fever. One month later this value had dropped to 34 mgm. per 100 cc. Although other tests for renal function were not done, it would appear that a transient decrease in renal reserve was induced by the attack of scarlet fever.

This patient was followed for 3 more years until her death on January 21 1938. In this period she had 3 infections one of which was shown to be Group A hemolytic streptococcus otitis media. During this epi-

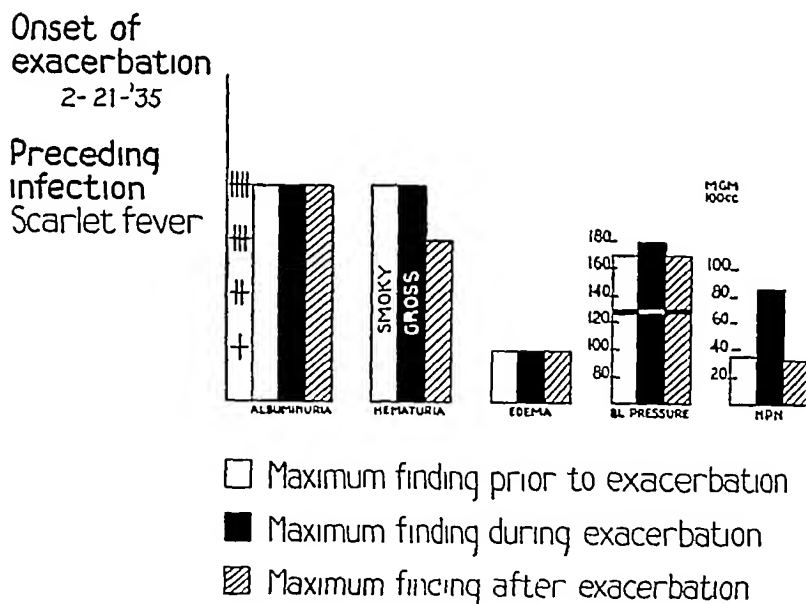


FIG 6 CASE VII (Wh) OBSERVATIONS BEFORE, DURING, AND AFTER EXACERBATION IN COURSE OF CHRONIC GLOMERULONEPHRITIS

sode the patient was followed outside the hospital and renal function tests were not performed. Death was apparently due to cardiac failure. Autopsy was not permitted.

Case VIII (Le)

This 14-year-old boy had a history of hematuria, malaise, and headache in July, 1938. There was no history of preceding infection, but when he was observed for the above symptoms he was found to have otitis media, and 1 week later he developed a sore throat. The albuminuria and hematuria diminished but persisted and he was admitted to the Presbyterian Hospital in September, 1938. Physical examination was negative except for a blood pressure of 140/90. The hemoglobin was 76 per cent, albuminuria varied from ++ to +++, and there were 1-30 red blood cells per high power field. The serum protein was 7.3 grams per 100 cc., the non-protein nitrogen 33 mgm. per 100 cc., and the phenol-sulphonphthalein excretion 55 per cent. There were no hemolytic streptococci on throat culture and the antistreptolysin titer was 71 units. The diagnosis was active glomerulonephritis.

In December, 1938, 3 days after exposure to "chilling," he developed gross hematuria. There was a history of a cough following the "chilling." Group A hemolytic streptococci were not found in the throat, but the antistreptolysin titer rose from a base line of 71 to 100 units and later fell to 50 units. Group F hemolytic streptococci were isolated from the throat on 3 occasions. As shown in the table below, there was no evidence for significant changes in renal function during this exacerbation.

	Before exacerbation	During exacerbation	After exacerbation
Hematuria	++	gross	++
Edema	0	0	0
Blood pressure	120/90	145/80	135/82
Nonprotein nitrogen	30	35	28
Urea ratio		50	40

In February, 1939, he developed gross hematuria one day after a head cold. No hemolytic streptococci were cultivated from the pharynx and the antistreptolysin titer did not rise. As shown in the table, the exacerbation caused no significant change in renal function.

	Before exacerbation	During exacerbation	After exacerbation
Hematuria	++	gross	+
Edema	0	0	0
Blood pressure	135/85	148/98	114/80
Nonprotein nitrogen	30	37	30
Urea ratio	42	46	50

In this patient 2 exacerbations occurring in a 3 month period produced no significant changes in renal function.

Case IX (Ke)

This 15-year-old boy was known to have had rheumatic fever and chorea at the age of 10. At that time his physician observed the development of an apical systolic murmur. In January, 1936, at the age of 12, he developed painless edema of his ankles and his blood pressure was elevated. However, a single urinalysis

was reported to show no abnormality at that time. The edema of the ankles was of short duration and he was in apparent good health until May 1937 when he had a severe sore throat with fever of 103. This was quickly followed by the development of erythema nodosum of both lower legs. Two weeks after his sore throat his urine became bloody and edema of the eyelids and ankles was noted.

He was first seen by us on July 9 1937 when he was believed to have subacute glomerulonephritis. Frequent urinalyses showed the presence of ++++ albumin with many red blood cells and a few granular casts in the sediment. The serum nonprotein nitrogen was 41 mgm per 100 cc. and the blood pressure of 135/90 at bed rest was considered slightly elevated for a boy of 13. The serum cholesterol was 312 mgm. per 100 cc. The only evidence suggesting active rheumatism was furnished by the electrocardiogram. The P-R interval rose from 0.16 to 0.20 and there were form changes consistent with the diagnosis of rheumatic myocarditis. These form changes disappeared within 3 weeks but the P-R interval remained 0.20 for the next 2 years.

This patient was observed closely in the following 2 years. In November 1938, he had a Group A hemolytic streptococcus throat infection without demonstrable exacerbation of nephritis or rheumatic fever. Of interest is the fact that the albuminuria which had been ++++ for 16 months, dropped to ++ with the onset of this Group A hemolytic streptococcus pharyngitis and persisted at this level for the next 3½ months until a fresh Group A hemolytic streptococcus infection appeared to increase the albuminuria to its previous ++++ level.

On March 25 1939 the patient developed a sore throat following coryza. Group A hemolytic streptococci were isolated from the throat 40 times in the next 2½ months. The antistreptolysin titer rose from a base line of 144 to a peak of 333 units. This was considered adequate evidence for hemolytic streptococcus infection. Within the next 2 months evidence for an exacerbation of the rheumatism rested on the development of a painful right wrist and an increase in the P-R interval from 0.20 to 0.24. In addition ST₁ and ST₂ were slightly elevated. The albuminuria which had been ++ for 4 months rose to ++++. In this same 4-month period the red blood cells in the urine had been reported as being absent or rare except for 2 of 9 examinations when the maximum value was one red blood cell per high power field.

From the Addis counts it was shown that the pre-micturition output of 3 000 000 white blood cells had risen to 75 000 000 after the pharyngitis. The excretion of red blood cells rose from 4 000 000 to 50 000 000 after the pharyngitis. The urea ratio which on 3 occasions between January 17 1939 and March 25 1939 had been between 29 and 31 rose to a maximum of 55 and subsequently dropped to 40. However the serum nonprotein nitrogen level showed no change. There was no edema, but the blood pressure reached a maximum of 180/120 with this episode. In the subsequent 3 months the blood pressure had decreased, but persisted at a higher level

than was observed in the pre-exacerbation phase. There were no demonstrable eye-ground changes. The phenol sulphophthalein excretion was always over 75 per cent.

It would appear that an exacerbation of rheumatic fever and glomerulonephritis occurred in this boy following a Group A hemolytic streptococcus infection. The evidence for a decrease in function associated with the nephritis is meager being based only on the rise in the urea ratio.

Case X (Ed)

This 17 year-old Negro was known to have had glomerulonephritis for at least 4 years previous to our first examination 8 months before his death. Early in life he was found to have congenital syphilis for which he received vigorous treatment. When seen by us his blood Kline test was strongly positive.

Early in October 1938, he developed coryza outside the hospital. This was quickly followed by the development of generalized edema with an increase in the degree of hematuria as compared with the previous values observed 2 months earlier. Although Group A hemolytic streptococcus was not recovered from his pharynx, his antistreptolysin level rose from 83 to 333 units.

The effect of this hemolytic streptococcus infection on the renal status of this patient is shown in Figure 7. The degree of albuminuria changed from ++ to ++++ and remained so until his death 4 months later. The degree of hematuria rose from + to ++++ with the exacerbation but then dropped to a minimum. The average Addis count for red blood cells in the prolonged post exacerbation period was 1 000 000 for the nocturnal 12 hour period. The changes in edema blood pressure nonprotein nitrogen retention, and phenolsulphophthalein excretion noted in Figure 7 indicate that, whereas the period of hematuria was associated with the development of marked edema, the other values were not strikingly abnormal in this same period. The patient was considered to have lapsed into the nephrotic state of chronic glomerulonephritis. The serum protein value was 4.0 grams per 100 cc. with the albumin 1.7 grams per 100 cc. The serum cholesterol was 305 mgm. per 100 cc. Although no further hemolytic streptococcus infections could be demonstrated there was a progressive drop in renal function beginning about 3 months after the onset of the exacerbation. The water retention became almost maximal, the blood pressure mounted to 230/130 and the serum nonprotein nitrogen level rose to 171 mgm. per 100 cc. The phenolsulphophthalein excretion which had been 40 per cent, dropped to zero per cent within the next 2 months.

The patient died on April 9 1939 of acute cardiac failure. The autopsy showed the renal lesions typical of chronic glomerulonephritis. Bismuth inclusions were found in the renal tubules. There was no histological evidence of acute glomerulonephritis.

From our study of this patient we were led to conclude that a hemolytic streptococcus infection had apparently induced an exacerbation of nephritis which at first assumed the pattern usually described as the ne-

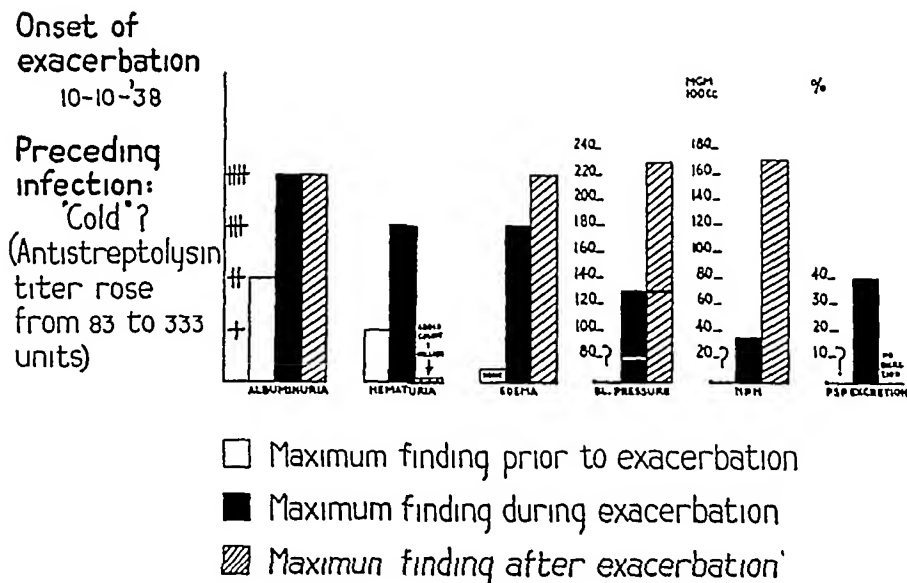


FIG 7 CASE X (Ed) OBSERVATIONS BEFORE, DURING, AND AFTER EXACERBATION IN COURSE OF CHRONIC GLOMERULONEPHRITIS

phrotic stage of chronic glomerulonephritis. It was not until 3 months after the onset of the exacerbation that a progressive decrease in renal function appeared independent of the disturbance in the salt-water equilibrium. It is possible that in this patient the hemolytic streptococcus infection initiated an exacerbation which resulted in a permanent drop in the renal function, but it cannot be denied that the progressive renal failure might have occurred in the absence of the infection.

Case XI (Pa)

This 17-year-old girl apparently developed acute glomerulonephritis in Puerto Rico at the age of 14. She gave a history of recurrent ulcers of both legs as a child but had never developed lymphedema of the extremities. It was of interest that the last episode of chronic ulceration of the legs terminated about 1 month prior to the onset of acute glomerulonephritis. The patient did not recall an upper respiratory infection prior to the onset of her initial attack of hematuria. During the next 2 years there were two attacks of gross hematuria, one in Puerto Rico and one in New York City. No data are at hand concerning prodromal infection with these episodes.

Two weeks prior to her admission to Presbyterian Hospital in May, 1939, she was said to have passed "red" urine. This persisted for 4 days. A few days after the onset of hematuria she was aware of a cold and 5 days later she had a sore throat with dysphagia.

When she was admitted to the hospital 1 week after the sore throat her pharynx was still red and her temperature was 101°. One out of 18 throat cultures showed a Group A hemolytic streptococcus. Her first antistreptolysin titer was found to be 500 units. This value rose to 1250 units and 4 months later had dropped to

250 units. The evidence would thus suggest that the exacerbation occurred concomitant with a hemolytic streptococcus infection.

From Figure 8 it is seen that the hematuria characteristic of the exacerbation is a transient finding. It is of interest that in this patient there is evidence that the exacerbation initiated a diuresis since the patient showed no edema during the acute phase of the renal flare-up in contrast to the data prior to and after the exacerbation. This finding is unrelated to salt restriction since water retention occurred in the post-exacerbation period despite the maintenance of a diet similar to that provided during the exacerbation. From the data on the chart concerning the phenolsulphonphthalein excretion and the serum non-protein nitrogen values, it is apparent that a transient drop in renal function occurred during the renal flare-up.

Case XII (Du)

This 18-year-old girl had been observed by us for 55 months prior to her death from uremia on November 29, 1938. At the age of 9 she had had scarlet fever with cervical lymphadenitis. In her tenth year she was reported to have had 3 severe sore throats, 2 of which were complicated by a peritonsillar abscess. A persistent albuminuria was detected about this time. At the age of 13 edema first appeared and the diagnosis of chronic glomerulonephritis was established.

She was first seen by us on April 19, 1934. In the following 55 months 2 frank exacerbations of the nephritis occurred. The first bout appeared one day after a mild sore throat on December 9, 1934. A hemolytic streptococcus was recovered from the pharynx but failed to grow on subculture when attempts at grouping were made. An adequate series of antistreptolysin determinations showed no rise above the previous base line of 25

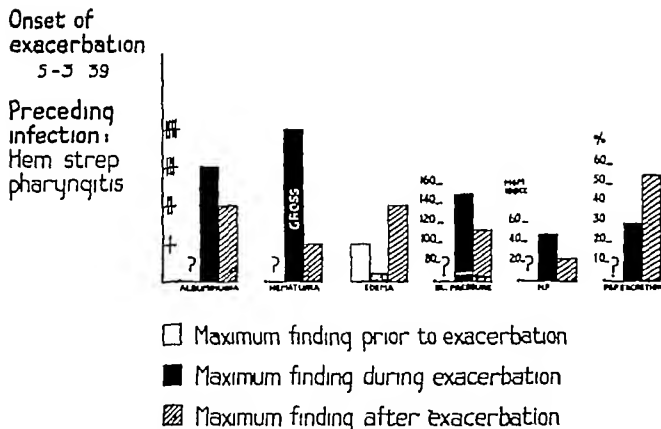


FIG. 8. CASE XI (Pa) OBSERVATIONS BEFORE, DURING, AND AFTER EXACERBATION IN COURSE OF CHRONIC GLOMERULONEPHRITIS

units. It was concluded, therefore, that there was insufficient evidence at hand to incriminate the hemolytic streptococcus as the cause of the exacerbation. The latter was characterized by gross hematuria. Urinalyses prior to the exacerbation had always shown many red blood cells in the sediment with some reports describing the sediment as saturated with red blood cells. The effect of this exacerbation on renal function was as follows. The minimal degree of edema remained unchanged. The pre-exacerbation blood pressure of 130/88 rose to 155/100 and the blood urea value rose from 30 to 47 mgm. per 100 cc. When the gross hematuria terminated, only 20 red blood cells per high power field could be found in each of 50 urine sediment examinations over a 2 month period. It was of interest, therefore, that the degree of hematuria immediately following the exacerbation was less than that observed in the pre-exacerbation period. Although the blood pressure continued at the exacerbation level blood urea determinations showed that a normal value was reached after the exacerbation.

The second exacerbation followed a prolonged hemolytic streptococcus infection beginning on April 13 1935. A sore throat associated with a shaking chill and high fever was followed by an acute cervical lymphadenitis and otitis media. These infections subsided without surgical intervention. Hemolytic streptococci were isolated from the pharynx 1 month after the onset of the sore throat. It was impossible to obtain earlier cultures since the patient was outside the hospital in a convalescent home. This infection was followed by a rise in the antistreptolysin titer from the base line of 33 units to a peak of 250 units.

Within 2 days following the onset of the sore throat, gross hematuria supervened. The bloody urine persisted for 5 days and then disappeared to return 4 weeks later following a recrudescence of the pharyngitis and cervical lymphadenitis. There was a temporary increase in the degree of edema and in the serum nonprotein nitrogen level during the exacerbation. The pre-exacerbation serum nonprotein nitrogen of 30 rose to 40 mgm. per 100 cc. during the renal flare-up but a subsequent blood urea determination in the post exacerbation period was within normal limits. Evidence was not obtained to prove a drop in renal function. It was of interest that the pre-exacerbation degree of hematuria of 80 to 100 red blood cells per high power field was decreased to 10 to 15 red blood cells per high power field following the second bout of gross hematuria. This lower value persisted for 2 months.

In the 3½-year period following the last exacerbation the patient was seen on 21 occasions. Seven colds and 2 sore throats occurred during this time. Group A hemolytic streptococci could not be recovered from the pharynx during these visits and the antistreptolysin titer was always between 25 and 50 units. The nephritis progressed, with microscopic hematuria and increasing hypertension the dominant features. Acute cardiac insufficiency manifested by dyspnea, even at bed rest, and nocturnal dyspnea characterized the final months of her illness. There was no edema. She died in uremia. Terminally there were convulsive seizures and pulmonary edema. The autopsy findings were those of chronic glomerulonephritis.

Case XIII (Mo)

This 33-year-old man was rejected for life insurance in 1933 because of obesity. Albuminuria and slight cylin-

duria without hematuria were demonstrated in 1936. In June, 1937, he developed pneumonia. Information concerning the etiology of this infection is not at hand. One week after the onset of the pneumonia gross hematuria appeared associated with renal colic. The blood pressure was 115/80 to 130/80 at that time. His physician ascribed this abnormality to renal calculus but subsequent x-rays failed to disclose the presence of a stone in the urinary tract. The pneumonia terminated and he was out of bed 3 weeks after onset. Except for epistaxis and frontal headache appearing about once a week, he was well for the next 6 months. Early in December, 1937, he developed an upper respiratory infection associated with cough and dyspnea. His headache and epistaxis became more severe and 1 week after the onset of the upper respiratory infection gross hematuria and edema developed.

He was first seen by us on December 29, 1937, 2 weeks after the onset of hematuria and the edema. The pharynx was injected but repeated throat cultures failed to reveal the presence of hemolytic streptococci. However, the antistreptolysin titer was 333 units. This level subsequently fell to 71 units. The latter data indicated a recent hemolytic streptococcus infection. During the first week in the hospital he was critically ill, being practically anuric for the first 24 hours. Figure 9 illustrates the available data concerning the urinary findings and certain functional tests in the periods before, during, and after the exacerbation.

It is seen that the gross hematuria during the exacerbation was reduced to a ++ level within 2 months. This degree of hematuria persisted unchanged for the next 19 months. The nonprotein nitrogen value of 175

mgm. per 100 cc during the exacerbation returned to a normal value of 30 mgm per 100 cc in 2 months and has persisted at this level. Similarly, the phenolsulphonphthalein excretion value of 3 per cent during the exacerbation, was 45 per cent in the fifth month, and 60 per cent in the eleventh month after the exacerbation. The edema disappeared following the exacerbation and the hypertension was markedly reduced. Almost 2 years after the exacerbation the only evidence for hypertension is noted in the diastolic phase where the values run between 87 and 105.

During the sixteenth month following the exacerbation, the patient experienced a severe sore throat. The antistreptolysin titer rose from the base line of 71 units to 200 units, though the few throat cultures which were available failed to reveal the presence of hemolytic streptococci. Gross hematuria did not supervene and the degree of albuminuria was unchanged. There were no significant changes in the renal function tests, all of which remained normal.

In summary, this 33-year-old man had apparently had chronic glomerulonephritis for at least 2 years before a pneumonia induced a gross hematuria. He was not observed by us through this episode. Six months later, however, a proved hemolytic streptococcus infection produced a major exacerbation of his nephritis. A profound decrease in renal function resulted, but additional study showed that this drop was temporary rather than permanent. It was of interest that 16 months following this exacerbation a hemolytic streptococcus pharyngitis failed to produce gross evidence of an exacerbation and did not impair renal function, which appeared normal by the usual tests.

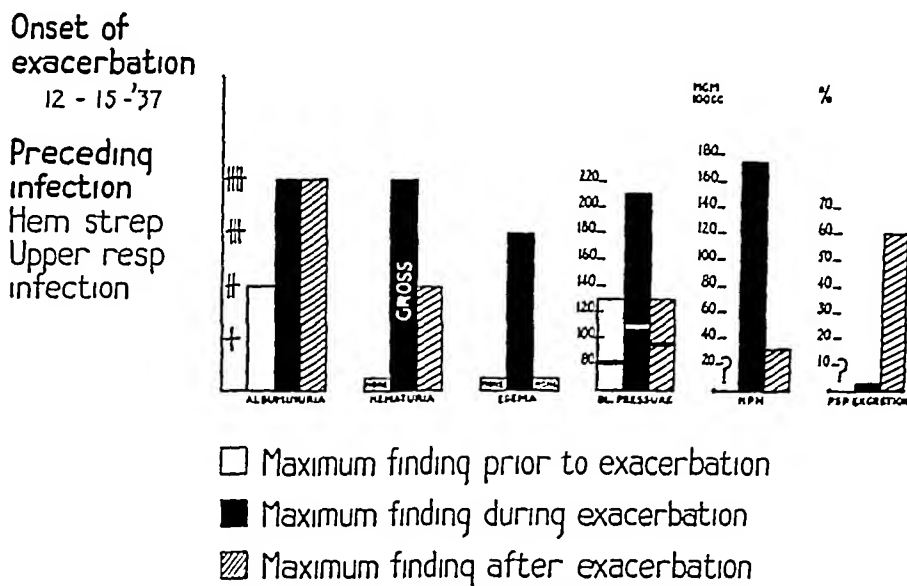


FIG. 9 CASE XIII (Mo) OBSERVATIONS BEFORE, DURING, AND AFTER EXACERBATION IN COURSE OF CHRONIC GLOMERULONEPHRITIS

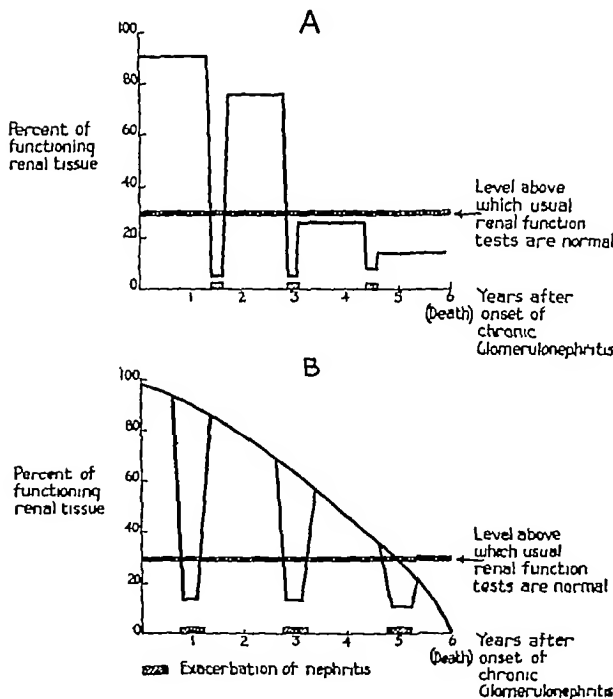


FIG. 10. SCHEMATIC DESCRIPTION (A AND B) SHOWING TWO POSSIBLE EFFECTS OF EXACERBATION ON MASS OF FUNCTIONING RENAL TISSUE IN HYPOTHETICAL PATIENT WITH CHRONIC GLOMERULONEPHRITIS

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STUDIES ON THE INTRAPULMONARY MIXTURE OF GASES

I NITROGEN ELIMINATION FROM BLOOD AND BODY TISSUES DURING HIGH OXYGEN BREATHING

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If a normal subject under resting conditions is changed from breathing atmospheric air to breathing 100 per cent oxygen, his lungs will then be progressively washed free of their normal content (about 80 per cent by volume) of the inert gas nitrogen, the latter being replaced by oxygen.

In addition, the nitrogen gas in physical solution in body tissues (in amount about 1000 cc.) will be gradually eliminated carried from the tissues to the lungs by the blood stream, and will finally diffuse into the alveolar air spaces which have been depleted of their nitrogen.

This procedure involves several physiological phenomena of interest in connection with pulmonary ventilatory function. The investigation of these will form the subject matter of this and the following papers. The phenomena concerned may be described as follows:

(a) The rate of emptying of nitrogen from the lung during pure oxygen breathing will depend upon a number of factors: the volume of residual air in the lungs, the volume of tidal air, rate of respiration, and the adequacy of distribution of each tidal breath to deeper pulmonary spaces. (1) This rate of emptying is also an effective means of measuring efficiency of the ventilatory process; that is, the emptying of nitrogen from a pulmonary air space with a given breath, gives an index of the effectiveness of this breath in removing carbon dioxide from, or adding oxygen to, this same air space.

The great defects that may exist in this function, in pathological lungs, can be illustrated by example. Figure 1 describes an experiment in which the nitrogen concentration of alveolar air has been measured during the course of pure

oxygen breathing (1) in a normal subject, and (2) in a patient with advanced pulmonary emphysema. The delay in emptying the lungs of nitrogen, in the latter case is striking. In the third paper of this series (2), we will return to a further consideration of this type of procedure.

(b) The nitrogen in the lungs has been found to serve as a convenient means of measuring lung volume. In the simplest and most widely used of these methods, that of Christie (3) the subject rebreathes for seven minutes in a closed circuit containing a spirometer filled with oxygen. The concentration of nitrogen in the lungs at the start of the procedure, the concentration in lungs and spirometer at the end, and the volume of the gases in the spirometer are known. From these values the volume of gas in the lungs can be calculated. The calculation assumes a nearly even mixture of nitrogen through the lung spirometer closed circuit at the end of the rebreathing period. In this case the lungs are emptied of a known fraction of the contained nitrogen rather than of all nitrogen. In the second paper of this series we attempt to study the adequacy of this assumption of even intrapulmonary mixture in normal and pathological subjects, in the Christie type of lung volume determination.

(c) In any method which attempts to measure intrapulmonary nitrogen by washing a part or all of this gas out of the lungs, it is obvious that a correction will be necessary due to the nitrogen excreted from the body into the lungs, whenever the normal alveolar nitrogen concentration is lowered. The present paper is concerned with the determination of an adequate correction factor due to nitrogen excretion from the body and its application in the measurement of residual lung volumes.

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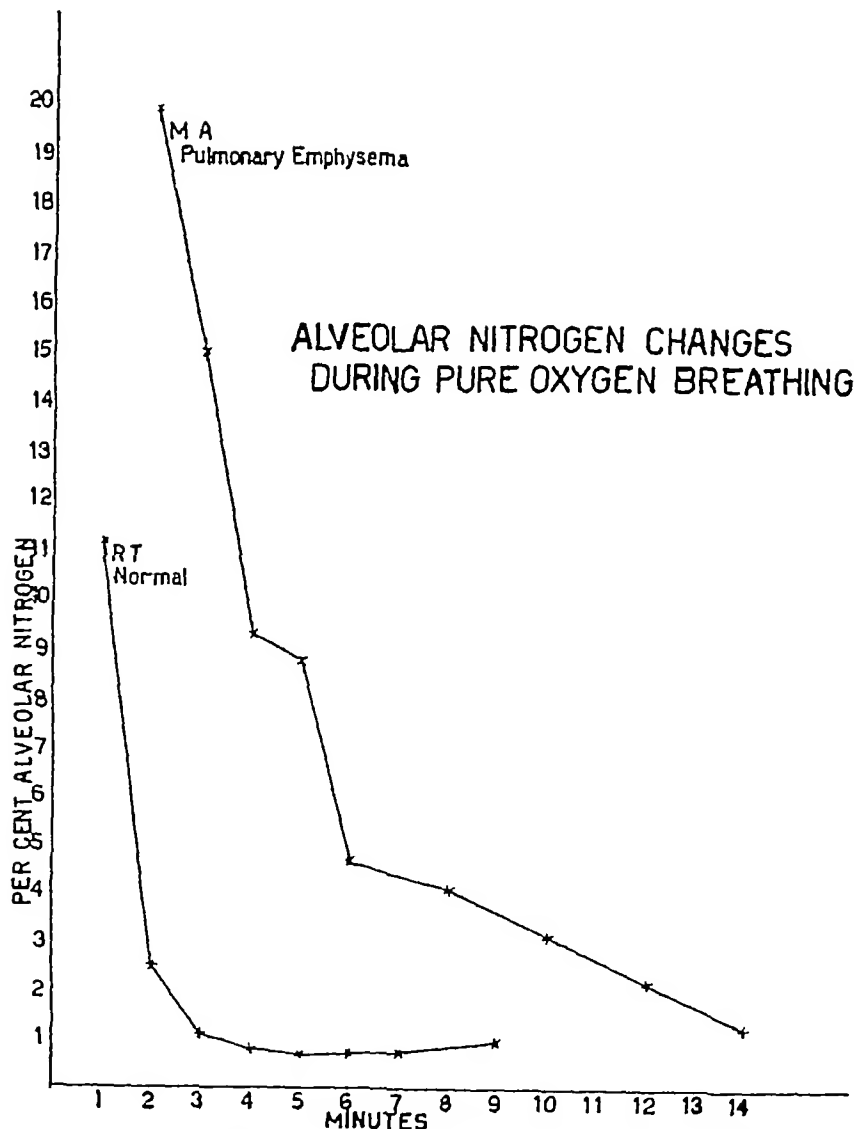


FIG 1 RATE OF EMPTYING OF NITROGEN FROM THE LUNGS DURING PURE OXYGEN BREATHING

Ordinates, per cent of nitrogen in alveolar air sample

Abscissae, time (in minutes) after starting pure oxygen breathing

The factors involved in the excretion of nitrogen (from the body into the lungs) may be listed as follows

1 Gradient of partial pressure of nitrogen between pulmonary blood capillaries and alveolar air spaces In normal subjects, as shown in Figure 1, alveolar nitrogen falls to a low figure within two minutes of oxygen breathing During this time the partial pressure of nitrogen in the tissues is still high, whereas the nitrogen in the lungs has been rapidly decreased by succes-

sive normal respirations The rate of excretion of nitrogen from the body will therefore be highest during the early minutes of oxygen breathing (after the first few breaths have established a blood-alveolar gradient), and will decrease as the tissue nitrogen is depleted

2 Since the alveoli are exposed to only a part of the circulating blood at any one time, the second important factor is the rate of blood circulation This principle has been used for estimations of cardiac output (4, 5), but has now

been discarded because of technical difficulties. Thus the nitrogen excretion should be lowest under basal conditions, i.e., when the cardiac output is at a minimum. It is useful to visualize the cardiac output as limiting the nitrogen excretion during any given period of time.

3 Time factor. This is simply the converse of the first two considerations, and its importance is sufficiently indicated above.

4 Total amount of nitrogen in the body. The size of the individual is obviously a significant factor. It was also shown many years ago by Vernon (6) later by Campbell and Hill (7), that a disproportionate part of body nitrogen exists in fatty tissues in which the gas is five times as soluble as in water or blood.

A careful study of nitrogen excretion from the body during oxygen breathing, as well as its reabsorption during nitrogen breathing has been carried out by Shaw and others (8), using dogs. They studied the process of nitrogen excretion only during the period *after* the residual lung volume had been washed nearly free of nitrogen, that is after the first seven minutes of quiet breathing. Their findings were (a) the curves of nitrogen excretion during oxygen breathing, and of nitrogen resaturation during air breathing after oxygen breathing, were similar (b) nitrogen excretion was proportional to the nitrogen pressure gradient between blood and lungs both facts predictable if the nitrogen is a simple dissolved gas.

For the calculation of a correction factor in lung volume determinations, it is of course the nitrogen excreted into the lungs during the first few minutes of oxygen breathing that is important. Several investigations of this aspect of nitrogen excretion have been made. In general the procedure has been to measure the total nitrogen eliminated from the lungs during a given period of oxygen breathing, then to subtract from this the excess of nitrogen in the lungs at the start of oxygen breathing over that still in the lungs at the end of the oxygen breathing period. It might seem that no satisfactory solution can be reached if the residual air must be known before nitrogen excretion can be calculated, and vice versa. Actually, the lungs can be nearly freed of nitrogen (down to 4 per cent)

by a few quick deep breaths, in this way any error in residual air figure will involve an error only 1/25 (4 per cent) as large in the final nitrogen excretion figure.

Using a small rebreathing circuit, Bornstein (4) found about 90 cc. of nitrogen excreted during the three minutes following a period of seventy seconds of overbreathing with oxygen to wash out the alveolar nitrogen.

Campbell and Hill (7) measured it for a similar initial three minute period and for subsequent two-minute periods up to fourteen minutes. Assuming but not measuring 60 cc. excreted during the first minute, they found values of 185 to 217 cc. during the first five minutes of pure oxygen breathing and 10 to 15 cc. per minute for later minutes. Their values during exercise were considerably higher.

Behnke, Thomson and Shaw (9) have measured nitrogen excretion in three normal men, using a technique similar to that of Shaw, Behnke, *et al* in their dog experiments. The nitrogen excretion during the first five minutes of oxygen breathing was not included. These investigators found that the curve of nitrogen excretion could be expressed by two equations, the first describing the early phase (first twenty five minutes) of nitrogen excretion, the second the later phase when nitrogen stores in body fat depots were being depleted. It is of interest that the time for complete unsaturation in man is longer than in dogs and that the two times are inversely proportional to the cardiac output per unit of body weight in the two species. Using the equation for the first part of their measured period, they have extrapolated to determine the curve for the first five minutes. From their data we have calculated the nitrogen excretion during the first seven minutes and found the figures to average 241, 181, and 212 cc. in the three normal subjects they studied.

In our experiments we have measured the nitrogen excretion only during the first seven minutes of oxygen breathing, since this is the period of interest in connection with lung volume measurement. In order to measure it during as much as possible of the first minute we have made the preliminary period for washing out the alveolar nitrogen only twenty seconds. This seems to be adequate for the normal subjects we

STUDIES ON THE INTRAPULMONARY MIXTURE OF GASES

II ANALYSIS OF THE REBREATHING METHOD (CLOSED CIRCUIT) FOR MEASURING RESIDUAL AIR

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The closed rebreathing circuit has been widely used for residual air measurement (1, 2, 3, 4, 5, and others). The essential features of the apparatus and procedure were the same in all. A spirometer filled with high oxygen (or with a hydrogen containing) gas mixture was attached to the test subject. The carbon dioxide was removed with soda lime or other alkali in the circuit and the subject breathed for a period of time until the nitrogen (or hydrogen) was redistributed in the circuit. The residual lung gas volume was calculated from the amount of redistributed gas.

In most of the work it was assumed that at the end of five or more minutes of quiet breathing the gas in lungs and spirometer was uniform in composition, except for a slight excess concentration of inert gases in the lungs. This excess was assumed to be the same as that occurring during the breathing of room air. On the basis of these assumptions the net change in spirometer gas concentration was considered the same as that in the lungs during the rebreathing.

By actual measurement of the gas concentration in the various parts of the spirometer system and the alveolar gas, Lassen, Cournand and Richards (6) concluded in the study of normal subjects that these assumptions were not valid. Rather than an excess they found a lower concentration of nitrogen in the lungs than in the spirometer at the end of a breathing period. They explained these findings as due to the constantly increasing nitrogen concentration which resulted from the steadily diminishing spirometer volume. This effect they called the 'oxygen storage' effect. To correct for it they introduced alveolar air concentrations into the calculation and found that this correction made a

difference of several hundred cubic centimeters in the residual air value in some subjects.

Other workers have found variable results in testing the need for this correction. Anthony (7) using a larger spirometer and a powerful motor blower failed to find any need for alveolar measurements. The discrepancy from Lassen, Cournand and Richards finding seemed to be explained by the difference in apparatus. Kaltreider, Fray and Hyde (8) believed from the study of six normal subjects that the correction due to the 'oxygen storage' effect was negligibly small. Hernald and McMichael (9) confirmed the presence of a significant 'oxygen storage' effect and proposed a modified procedure in which the spirometer volume was kept constant by added oxygen.

In severe cases of pulmonary emphysema, Cournand, Lassen and Richards (10) found no such predictable 'oxygen storage' effect as in normal subjects. Moreover they found extreme variability of results in these subjects on successive measurements. Some figures obtained seemed improbably high, considering the external chest measurements of the subjects. From this they suggested that in these subjects there was an unequal distribution of respiratory gases within the lungs. If this were true the alveolar air as measured was not a true index of average lung gases. Previously Sonne and his coworkers (11, 12, 13) had shown this lack of uniformity of the alveolar air not only in emphysema but also in some normal subjects. By fractional analyses of alveolar air after breathing hydrogen Roelsen (14, 15) has shown an even greater factor of unequal distribution than was evident in analyses of the normal respiratory gases. In the residual air measurements it is possible that this factor may be still more magnified in its effect, since here the entire gaseous

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hand, gives no information as to the true value for the residual lung volume

METHODS

Method Ia (decreasing lung nitrogen, diminishing volume)

This is the Christie method as modified by Lassen, Cournand, and Richards (6) to include alveolar air measurements before and at the end of the rebreathing period. The apparatus shown in Figure 1 is fundamentally that of a Benedict-Roth basal metabolism apparatus, with a few additions. The soda lime carbon dioxide absorber (*S.L.*) and the flutter valves (*F*₁, *F*₂) are inserted outside the spirometer. The chief addition is a valve (*V*₁) adjacent to the mouthpiece (*M*), which can be turned either into the spirometer circuit or into a side circuit fitted with similar flutter valves (*F*₃, *F*₄). In the expiratory side of this circuit are inserted the alveolar sampling tubes. The dead space from mouthpiece to these tubes is approximately 100 cc. A valve (*V*₂) is inserted to close the inspiratory gas flow during alveolar sampling.

In preparing the apparatus for Method Ia, the dead space was washed repeatedly with room air by raising and

lowering the spirometer bell. The water level in the spirometer was kept at a measured level. At this level the dead space of the spirometer system had been previously determined (Christie). After washing, the valve (*V*₁) was turned to the side circuit, thus closing the spirometer circuit. Oxygen was then admitted through *R I*, usually about 4000 cc. The actual amount was measured on the graphic tracing after constant temperature had been reached. In practice, the amount of oxygen added was varied, depending on the size of the residual air and the oxygen consumption expected. Ideally, it should be such an amount that the final spirometer gas after rebreathing should be slightly under 50 per cent oxygen. The apparatus was tested for leaks by registering a short period on the drum with a weight placed on the spirometer bell. A straight horizontal line should be drawn.

The subject, preferably under basal conditions, then inserted the mouthpiece and applied the nose clip. When breathing quietly through the side circuit, he was instructed to exhale fully for an alveolar specimen which was taken at the end of three to five seconds of forced expiration. Quiet breathing was then resumed and continued one to two minutes, or until the effect of the exertion of alveolar sampling had passed. With the drum moving,

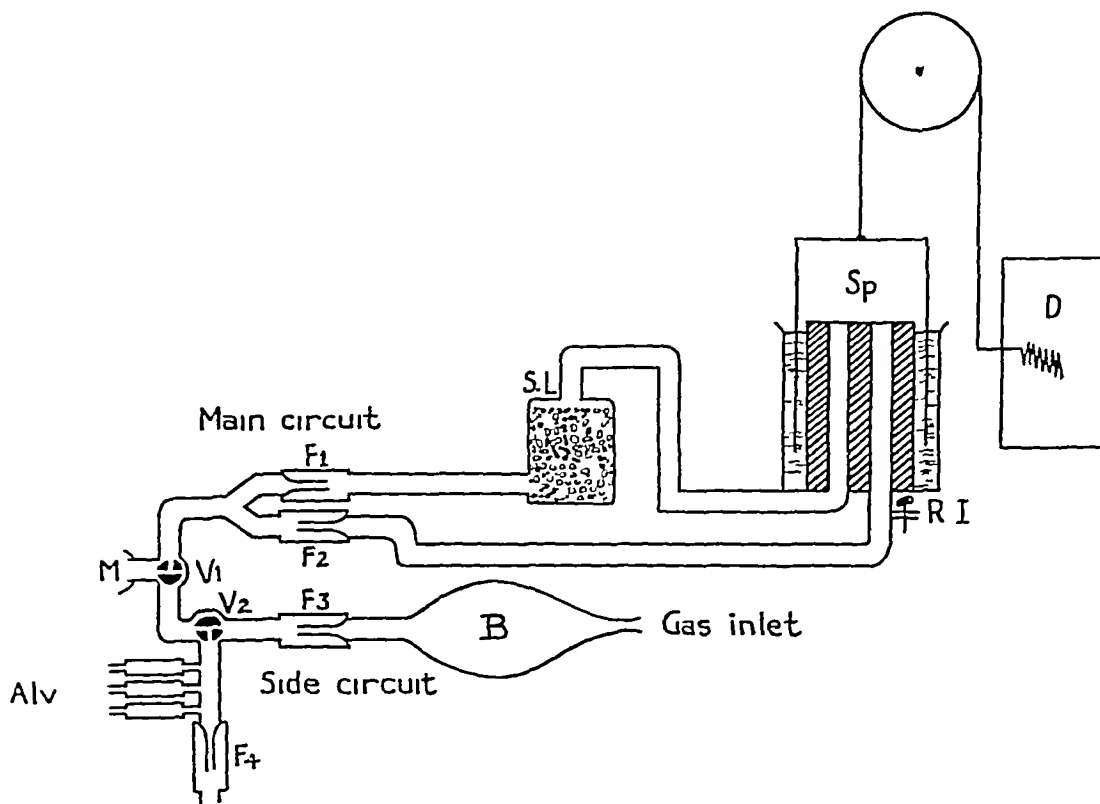


FIG 1 DIAGRAM OF CLOSED CIRCUIT APPARATUS FOR ANALYSIS OF INTRAPULMONARY MIXTURE OF GASES

M, mouthpiece *Alv*, set of three evacuated gas sampling tubes *V*₁, *V*₂, three-way respiratory valves *F*₁, *F*₂, *F*₃, *F*₄, one-way rubber flutter valves *B*, rubber anesthesia bag *S.L.*, soda lime absorber for CO₂ *Sp*, spirometer *R.I.*, side valve for sampling spirometer gases *D*, recording drum

the valve (V_1) was then turned to the spirometer circuit as nearly as possible at the end of a quiet expiration. Quiet breathing was recorded for the standard time of seven minutes, or a longer period as desired in some data to be reported. Near the end of this period the spirometer temperature was read. At the end of this time the valve (V_1) was turned to the side circuit and simultaneously the subject expired fully for alveolar sampling. It was not necessary that this valve shift occur at the end of a normal expiration. In fact it was found better to turn it immediately after the start of the expiration. The spirometer gas sample was then taken by placing a weight on the spirometer bell opening *R.I.* allowing approximately half the gas to escape and then taking the sample.

There were three gases to be analyzed: the spirometer and two alveolar specimens which we shall call $\bar{\alpha}$ and $\bar{\beta}$. For calculation only the nitrogen percentage needed to be measured as the difference between the total and the combined oxygen and carbon dioxide. Actually frequent analyses were made for carbon dioxide separately on the spirometer to check the efficiency of the soda lime and on the alveolar specimens to be sure that specimens were not significantly diluted with dead space gas. Analyses were made with a Haldane apparatus in which the burette was calibrated for up to 50 per cent absorbable gases.

All volume measurements were made from the tracings, together with the previously measured dead space of the apparatus. The volume of added oxygen in the spirometer was recorded at the time it was run in: the oxygen consumption during the experiment was measured from the slope of the tracing. The initial volume minus this consumption equals the final spirometer volume to which the dead space volume was added to obtain the final volume of spirometer system.

The formula for the F.R.A. calculation is obtained from a mathematical statement that the nitrogen in the lungs and spirometer at beginning and end is equal (with a correction for nitrogen excretion).

Let $V\bar{\alpha}$ = vol. N_2 containing gas in spirometer system at start (in this case = D.S. dead space)

$V\bar{\beta}$ = final spir. vol. + D.S.

Spir. $\bar{\beta}$ = analysis of spir. gas for N_2 expressed as part of an atmosphere

Alv. $\bar{\alpha}$ } = alveolar N_2 analyses in same units.
Alv. $\bar{\beta}$ }

F.R.A. = functional residual air in cc.

Then

F.R.A. (alv. $\bar{\alpha}$) + $V\bar{\alpha}(0.791)$

= F.R.A. (alv. $\bar{\beta}$) + $V\bar{\beta}(\text{Spir. } \bar{\beta}) - N_2 \text{ excretion}$

Solving

$$\text{F.R.A.} = \frac{V\bar{\beta} \text{ Spir. } \bar{\beta} - V\bar{\alpha}(0.791) - N_2 \text{ excretion}}{\text{alv. } \bar{\alpha} - \text{alv. } \bar{\beta}}$$

From Paper I of this series (16)

$$N_2 \text{ excretion} = \frac{\text{alv. } \bar{\alpha} - \text{alv. } \bar{\beta}}{0.80} \times 220$$

(For seven minutes breathing time 10 cc added to 220 cc for each minute after seven if longer period)

Substituting

$$\text{F.R.A.} = \frac{V\bar{\beta} \text{ Spir. } \bar{\beta} - V\bar{\alpha}(0.791)}{\text{alv. } \bar{\alpha} - \text{alv. } \bar{\beta}} - 275$$

All analyses with Haldane apparatus give proportions of dry gas so $V\bar{\beta}$ and $V\bar{\alpha}$ were corrected to dry gas at the temperature of the experiment before substituting in the formula. The F.R.A. value then obtained may need a slight correction if the rebreathing period did not begin at the exact end of a normal expiration. Such a correction was determined from examination of the tracing. After this correction the F.R.A. volume was corrected to 37°C and saturation with water vapor.

Method Ib (decreasing lung nitrogen, constant volume)

This procedure differed only in a few details from Ia. Immediately after the start of the rebreathing period a steady flow of oxygen was begun through *R.I.* (Figure 1) equal to the resting oxygen consumption. This figure was estimated from a previous tracing. For regulating the flow a Forreger flow measuring valve was used with a fine and coarse water manometer gauge. This flow of oxygen was turned off fifteen seconds before the end of the rebreathing period.

The formula for calculation is unchanged. $V\bar{\alpha}$ is still the D.S. (dead space). A base line drawn on the tracing as in Ia will be approximately horizontal. $V\bar{\beta}$ will be D.S. + O_2 volume added at start \pm correction for any deviation of tracing from horizontal.

Method IIa (increasing lung nitrogen diminishing volume)

This method required the same apparatus with only one addition. On the inlet valve of the side circuit a rubber anesthesia bag (B) was attached. This in turn was connected with an oxygen tank fitted with reduction and flow measuring valves.

In preparing the apparatus, the spirometer and dead space were washed with room air as before then partly filled with room air and tested for leaks. The volume of room air admitted to spirometer was usually 2000 to 3000 cc. but was conveniently made larger with subjects of large F.R.A. and smaller with small subjects.

With V_1 open to the side circuit and the oxygen flow in that circuit maintained at 7 to 8 liters per minute the subject was then attached to mouthpiece and allowed to breathe quietly for ten minutes. Oxygen flow was adjusted to keep the anesthesia bag partly full. The ten minute period was chosen because preliminary experiments measuring alveolar nitrogen values at frequent intervals during oxygen breathing showed that in normals a low plateau level for alveolar nitrogen was reached after three to four minutes in severe emphysema a slightly higher plateau level was reached in ten to twelve minutes.

At the end of ten minutes of oxygen breathing an alveolar specimen was taken with the inlet valve (V_2) closed for the few seconds of the procedure. Oxygen breathing was continued for two additional minutes to reach a resting level then at the end of a normal expiration

valve V_1 was turned to the spirometer system. For seven minutes or longer, spirometer breathing was recorded on the drum and an alveolar specimen taken at the end, as in Method I. It was found advisable to partly wash out the tubing of the side circuit with room air before taking the alv p specimen, in order that the high oxygen previously there would not too greatly dilute the nitrogen of the alveolar air.

Using the same symbols as in Method I, the calculation formula is similarly derived

$$F R A (alv \bar{a}) + V\bar{a}(0.791)$$

$$= F R A (alv \bar{p}) + V\bar{p}(\text{Spir } \bar{p}) + N_2 \text{ absorbed}$$

Solving

$$F R A = \frac{V\bar{a}(0.791) - V\bar{p}(\text{Spir } \bar{p}) - N_2 \text{ absorbed}}{alv \bar{p} - alv \bar{a}}$$

Substituting formula for N_2 absorption

$$F R A = \frac{V\bar{a}(0.791) - V\bar{p}(\text{Spir } \bar{p})}{alv \bar{p} - alv \bar{a}} - 275$$

$V\bar{a}$ in this case is the dead space plus the room air added to system. $V\bar{p}$ is this value minus the oxygen absorbed during the rebreathing period. Corrections are made as in Method I for inexact starting point, temperature and for water vapor.

Method IIb (increasing lung nitrogen, constant volume)

This modification of Method II is exactly analogous to the "b" modification of Method I. The volume was kept nearly constant by a steady flow of oxygen into $R I$ during the rebreathing period, up to fifteen seconds before the end.

The calculation formula is likewise unchanged. $V\bar{p}$ in this case equals $V\bar{a} \pm$ any necessary correction for deviation from horizontal in the slope of the tracing.

Actually the Method IIb was used more frequently than IIa in those cases in which only a comparison of results by Methods I and II was desired. One reason for this was that in the "b" modification there is no chance of obtaining a final breathing mixture of less than 21 per cent oxygen, as is possible in IIa.

RESULTS

The subjects for this work consisted of six normal persons and ten patients with severe pulmonary emphysema. The normal subjects included physicians and ambulant hospital patients with non-pulmonary diseases. In the patients with emphysema, the diagnosis was established by the physical signs, the x-ray and the spiographic tracings. All suffered from severe dyspnea, six out of ten showed a reduced arterial oxygen saturation. Only one subject (Ant C) seemed to be suffering from some degree of cardiac failure, and in this case the pulmonary disturbance seemed predominant. Two subjects who have since died

were examined at autopsy and the diagnosis of emphysema confirmed. They also showed some degree of bronchiectasis and it is probable that some of the living subjects also showed this common complication of advanced obstructive emphysema. The group of abnormal subjects is therefore a picked group, chosen not as average cases but as extreme and advanced cases.

Table I shows the results on the entire series by Methods Ia and IIb. In addition, calculated figures for the Ia experiments are listed, using

TABLE I
Functional residual air determinations by closed system

NORMAL SUBJECTS						
Subject	Length of test	Decreasing lung N ₂ method—Ia			Increasing lung N ₂ method—IIb	
		Number of determinations	Christie calculated average	Lassen <i>et al</i> calculated average	Number of determinations	Average
J D	minutes	3	1225	1115	3	1065
J L	7	12	1420	1320	12	1300
	12				3	1210
A C	7	2	1895	1745	3	1770
	10				1	1735
T R	7	4	5500	4375	3	4460
	10				1	4300
D W R	7	3	4040	3540	2	2930
	10	1		3470	1	3310
R C D	7	5	3990	3250	6	2330
	10	2		3810	2	2340
Mean of group			3010	2560		2310
S D			1740	1480		1370
S E			710	602		503
<i>t</i> *			0.5		0.3	

SUBJECTS WITH PULMONARY EMPHYSEMA						
M K		4	4100	3320	4	2390
J O		5	5180	4380	4	2530
M H		15	6060	5360	4	3845
J F	7	5	4760	4240	4	2280
	12	1		5040	2	2085
M A		7	7490	5465	9	3320
Ant C		2	3490	3240	2	2850
J C	7	4	7210	6570	4	5430
	12	2		6285	2	5470
D H		2	3365	3140	2	2530
H K	7	3	3790	3835	3	2250
	12	2		3765	2	1940
F H		1	3160	3020	1	1945
Mean of group			4860	4260		2960
S D			1680	1270		1120
S E			531	401		354
<i>t</i> *			0.9		2.4	

* $t = \frac{\Delta_m}{S E \text{ Difference of Means}}$

the original Christie calculation and neglecting the alveolar measurements. The average value of a series of determinations is given for each of the three calculations. Results of Methods Ib and IIa are omitted from this table but will be presented in a later detailed listing of results on four subjects, together with an analysis of the variations by each single method.

A comparison of columns 1 and 2 gives confirmation to the findings of Lassen, Cournand and Richards (6) on the influence of alveolar measurements in the calculation. Of the six normal subjects the Christie calculation gave larger results in every instance, the difference varying from 100 to 1125 cc. Of the ten emphysematous subjects, nine showed higher results by the Christie calculation, the difference varying from 140 cc. to almost 2000 cc. In one (H K) the Christie calculation gave practically identical results.

A comparison of columns 2 and 3 gives the evidence by which the factor of unequal distribution in the lungs may be discovered and roughly quantitated.

Normal subjects

Of the six normal subjects the two methods give practically identical results in four. Of these four, three were subjects with functional residual air of less than 2000 cc. In the case of the other two normal subjects both with rather large functional residual air there was a difference of 600 cc. and 900 cc. respectively in the average values by the two methods. It should be mentioned that no abnormalities could be found clinically or by x ray of the chest in these two subjects. Thus it would seem that in these two normal subjects there was indirect evidence that the alveolar air as measured does not indicate the gas concentration throughout the lungs under the conditions of the experiment.

Theoretically this situation might be corrected by increasing the rebreathing time beyond the usual seven minutes. Such an experiment was carried out on these two subjects. In a single pair of ten minute tests on one of them there appeared to be better agreement than in the seven minute tests. In the case of the other subject, however no closer agreement could be reached.

Subjects with emphysema

In the experiments on the emphysematous subjects it will be seen from Table I that there was in every instance a significant difference between average results by Methods Ia and IIb. This difference ranged from 400 cc. in the case of Ant. C to 2000 cc. in the case of J F. In seven out of ten cases, the difference was greater than 1000 cc. It would seem that these findings have similar significance to those in the case of the last two normals, except that the degree of change is more marked in the pathological cases. Evidently the alveolar air samples as obtained were in all cases a poor measurement of the average lung gases even though the alveolar carbon dioxide level in the gases analyzed was regularly above 5 per cent. The comparable findings in some normal subjects and all emphysematous subjects would seem to follow closely Sonne's findings of variations in different parts of the measurable alveolar air in the same groups of subjects.

As in the case of the normal subjects, an attempt was made to repeat the experiments, using longer breathing periods. Such experiments are technically somewhat more difficult. In three cases results of a twelve-minute breathing period are presented in the same table (Table I). It will be seen that in no case was agreement obtained between the two values. Furthermore there is no definite trend toward decreasing the difference between them. In two there is actually an increase in the difference, in the third a slight decrease probably not significant.

Table I also shows a statistical analysis of the seven minute figures by the various methods. Such an analysis of small groups of subjects, however introduces a large variable in the size of the chest within the group so that the tests of significance between the different methods are no measure of significant differences due to the method alone. In spite of this unfavorable type of comparison it will be seen that in the group of abnormal subjects there is a statistically significant difference between the means of results by Methods Ia and IIb.

To obtain a comparison of the groups in which only the factor of difference of method is involved a second table is presented (Table II), expressing only the relative values by each

TABLE II

Relative mean values of functional residual air by closed circuit methods

NORMAL SUBJECTS			
Subject	Decreasing lung N ₂ method—Ia		Increasing lung N ₂ method—IIb
	Christie calculation	Lassen <i>et al</i> calculation	
J D	1 10	1 00	0 96
J L	1 07	1 00	0 98
A C	1 09	1 00	1 02
T R	1 26	1 00	1 02
D W R	1 14	1 00	0 83
R C D	1 23	1 00	0 72
Mean	1 15	1 00	0 92
S D	0 087		0 133
S E	0 034		0 054
t		4 4	1 5
SUBJECTS WITH PULMONARY EMPHYSEMA			
M K	1 24	1 00	0 72
J O	1 18	1 00	0 59
M H	1 13	1 00	0 72
J F	1 12	1 00	0 54
M A	1 37	1 00	0 64
Ant C	1 08	1 00	0 88
D H	1 21	1 00	0 81
J C	1 10	1 00	0 82
H K	0 99	1 00	0 59
F H	1 05	1 00	0 64
Mean	1 15	1 00	0 70
S D	0 115		0 121
S E	0 036		0 038
t		4 2	7 9

method, arbitrarily setting the value by Method Ia as unity in each subject. In this comparison it will be seen that the Christie calculation gives significantly higher values in both groups. Considered as a group, Methods Ia and IIb are not significantly different among the normals. In the abnormal group, the striking difference of the two methods is clearly shown.

Table III presents the more complete data on four subjects studied thoroughly: one normal subject with small residual air, one normal subject with large chest who showed discrepancies between results by the contrasting methods, and two subjects with pulmonary emphysema. Such a comparison is more valuable than analysis of groups since the subjects were picked as strikingly abnormal cases and, also, the evaluation of any method may depend on its applicability to the extreme and unusual case.

In the case of J L, a small normal subject, it is evident that the results by all the methods using alveolar measurements are not significantly different. Even here, however, the Christie calculation is significantly higher than that using alveolar measurements. It should also be noted, in this small subject, that there is much less variability of results by the same method than in the other three subjects.

The tabulation on R C D merely repeats in statistical form the results on this subject.

TABLE III

Functional residual air values by closed circuit methods, statistical analyses on individual subjects

	J L (Normal)				R. C D (Normal)				M H (Emphysema)				M A (Emphysema)			
	Num ber of deter minations	Mean	S D	S E	Num ber of deter minations	Mean	S D	S E	Num ber of deter minations	Mean	S D	S E	Num ber of deter minations	Mean	S D	S E
Method Ia																
Christie Calculation	12	1420	105	30	5	3990	442	198	15	6060	821	212	7	7490	1740	660
Lassen <i>et al</i> Calculation	12	1320	108	31	5	3250	359	160	15	5360	748	193	7	5465	553	209
Method Ib	6	1290	48	35					5	4630	105	47	5	4560	373	167
Method IIa	8	1230	82	29					8	3865	368	130	10	3475	170	54
Method IIb	15	1270	79	20	6	2330	221	90	4	3845	316	158	9	3320	196	65
Values compared	t				t				t				t			
Christie Calculation vs Lassen <i>et al</i> Calculation	2.3				2.9				2.4				2.9			
Ia vs Ib	0.6								3.7				3.4			
IIa vs IIb	1.1								0.1				1.8			
Ia vs IIb	1.3				5.0				6.1				10.2			
Ib vs IIb	0.5								4.9				6.3			

in Table I. It will be seen that the comparison to show the effect of "oxygen storage" and also the comparison to show the possible effect of poor mixing both give statistically significant differences.

The figures on M A and M H are not only an analysis of those in Table I, but also include figures by Method Ib (which is essentially the modification of McMichael) and by Method IIa. The results by Methods Ia and IIb are widely divergent, as is evident from inspection. A comparison of the values by Ia against Ib and by IIa against IIb will show how far the maintenance of constant volume corrects the error due to imperfect intrapulmonary gas mixture. In these two subjects the values by Ib were significantly lower than by Ia, yet there is even more difference between either of these and Method II. Further more, in the case of 'a' and 'b' modifications of Method II, there is no significant difference.

The findings in these two subjects are characteristic of several others so studied. In no case did the maintenance of constant volume remove the evidence for error due to admixture within the lungs.

DISCUSSION

These data not only give us some information on the limitations of the rebreathing methods for measuring residual air, especially in severe cases of emphysema but they also furnish an indirect measure of imperfect gas mixture in the lungs. The reversed Christie procedure. Method II was devised in such a way that, if uniform mixture within the lungs occurred, the two methods should give identical results. Furthermore the probable direction of the error in case of imperfect mixture was predicted in each method assuming that there was gas trapped in the lungs from previous breathing which did not contribute to the alveolar air measured.

The uniform evidence of unequal distribution in the emphysematous subjects is not surprising. It has long been evident that such might be the case from the anatomical changes in the lungs of such subjects. Analysis of gas obtained by direct puncture from large emphysematous bullae has shown very high nitrogen values (17). Sonne's fractional analyses of an alveolar sample showed directly a marked variability. Roelsen repeated the fractional alveolar analyses following a single breath of hydrogen and deduced from

the results that the mixture was even more imperfect than was evident from the carbon dioxide and oxygen analyses alone. This latter finding with the use of hydrogen would seem to give the clue to the reason why the factor of maldistribution has been so rarely appreciated. The fractional analyses of alveolar air for carbon dioxide and oxygen give evidence of unequal distribution only if the poorly aerated lung regions are relatively well perfused with blood. If in the course of disease the blood circulation in those areas diminishes parallel to the drop in aeration then the alveolar samples may be uniform and the alveolar carbon dioxide tension may correspond well to that in the arterial blood. This correspondence between alveolar and arterial blood carbon dioxide tensions has been an important piece of evidence and has been interpreted to signify uniformity of gas mixture within the lungs.

In spite of the previous evidence that gas mixture is slow in emphysematous lungs, it has been assumed that the seven minute period was adequate to overcome difficulties arising from this slow mixture. It seems likely from our data that the factor of poor mixture in the lungs is an important source of error not only after seven minutes breathing but also after ten to twelve minutes. It seems doubtful whether increasing the time further would remove the difficulty. Furthermore, the magnitude of the discrepancies due to poor mixture in our data seems to indicate that this factor is a major one not only for the measurement of residual air, but probably also for the general problem of the pulmonary disturbance.

The finding of a factor of imperfect distribution in certain normal subjects was somewhat more surprising. Sonne's work showed that it existed but not that it was as large as the data here indicate. Possibly our method is sensitive to detect small degrees of maldistribution.

It is evident that our data give no conclusive evidence as to the true residual air value in the cases showing different results by the various methods. The residual air volume has been considered an important index in emphysema of the degree of disability usually expressed in relation ship to the vital capacity or the total capacity. It is possible in these cases that the residual air value, as measured by the Christie method is not a true volume measurement, but represents

the true volume plus an added value due to the factor of poor gas mixture. In order to prove this, it would be necessary to have another method for comparison which minimizes or eliminates the effects of mixing.

A detailed consideration of the rebreathing method has shown why, in cases of poor mixing, the chances of true equilibrium between lungs and spirometer are not good. Even keeping the volume constant as in McMichael's work or our Method Ib, there is a constantly changing breathing mixture for the first few minutes of the rebreathing. If the volume is diminishing, the changes are more complicated and the inspiratory gas mixture is always changing. Furthermore, the size of the usual spirometer is such that the change in alveolar nitrogen is usually 0.2 to 0.3 atmosphere. In the course of calculation, such a small change leads to a three- to five-fold magnification of error in the residual air value.

Thus, a method which would allow a uniform inspiratory gas mixture and would utilize a maximum alveolar change should offer greater likelihood of reaching a true measure of residual air. Such a method will be presented in Paper III of this series (18).

SUMMARY AND CONCLUSIONS

1 Modifications of the Christie method of residual air measurement are presented which (1) keep the spirometer volume constant, (2) reverse the shift in nitrogen and therefore reverse the direction of error due to unequal distribution within the lungs.

2 Results are presented using the different modifications and the original method on six normal and ten subjects with pulmonary emphysema.

3 These results give agreement by all methods in four normal subjects only, three of whom had small residual air volume.

4 In the remainder of the normal subjects and all the subjects with emphysema, there are wide discrepancies between the results by the different methods. Keeping the spirometer volume constant does not correct the discrepancies. These data give positive evidence of unequal gas distribution within the lungs.

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STUDIES ON THE INTRAPULMONARY MIXTURE OF GASES

III AN OPEN CIRCUIT METHOD FOR MEASURING RESIDUAL AIR

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In the preceding paper, the use of the closed rebreathing circuit for measuring residual air was discussed and tested to determine the possible error due to imperfect gas mixture within the lungs. It was found that such an error was probably present not only in all cases of severe pulmonary emphysema, but also in some normal subjects with large residual air. Some of the reasons for the inadequacy of the closed circuit measurements are apparent from analysis of the essential features of such a circuit. In the first place, the inspiratory gas mixture is always changing. Thus there would appear to be no sustained equilibrium between spirometer and lungs. As soon as equilibrium is approached for one concentration of the breathing mixture the inspiratory gas has already changed. This change is most marked when the spirometer volume is allowed to diminish as oxygen is absorbed. McMichael's technique of replacing the oxygen, as tested by us, did not remove the discrepancies due to poor mixing within the lungs. Furthermore, such a procedure introduces further technical difficulty and fails to change the fact that the inspiratory gas is still varying at the start, until approximate equilibrium is reached. In our experience, the adjustment of a proper flow of oxygen for replacement was difficult even in normal subjects. In subjects with arterial oxygen unsaturation the maintenance of a constant volume in the closed circuit was practically impossible due to the oxygen deficit and resultant changing rate of oxygen replacement necessary.

The second difficult feature of closed circuit measurements is the exact calculation of the oxygen absorbed. The tracings in abnormal and

even in some normal subjects are often so irregular that an exact base line cannot be drawn, yet the calculation demands accuracy in this detail.

Thirdly, with the usual closed circuit technique the net change in lung nitrogen concentration is rarely more than three-tenths of an atmosphere. With such a figure, any error in alveolar measurement (or in the assumed values) is magnified at least three fold in the final residual air value.

To avoid these three points of difficulty, an open circuit method has been devised, with pure oxygen as the breathing mixture. In such a procedure, the subject is allowed to breathe oxygen for a period of time sufficient to wash practically all the nitrogen out of the lungs. For this period all the expired gases are collected and finally measured and analyzed for nitrogen. It will be seen that the inspiratory gas is absolutely uniform throughout and that there is no need to obtain a smooth breathing curve once the oxygen breathing has been started at a definite point in the breathing cycle. As in the closed circuit, the concentrations of gases in the pulmonary spaces at start and end are estimated by alveolar specimens, yet here the simpler features of the procedure make these measurements less subject to error. Furthermore, since the net change in alveolar nitrogen is approximately eight tenths of an atmosphere, the effect of errors in these measurements will not be greatly magnified in the course of calculation.

Thus the only errors that are to be anticipated in this method are those due to failure of the alveolar measurements to represent the mean value of residual air nitrogen. It is possible to predict the probable direction of such error. Except in unusual circumstances the alveolar specimens in cases of poor distribution will

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to represent the well aerated portions of the lungs, and to neglect the relatively poorly aerated regions. Thus, in estimating the lung nitrogen concentration on room air breathing at the start, the alveolar specimen obtained may be lower in nitrogen than the average in the lung. Similarly, the alveolar specimen after a period of oxygen breathing may fail to tap some nitrogen still present in the poorly aerated regions and so be somewhat too low as measured. If breathing is continued long enough, the latter error should be gradually reduced, since it is obvious that eventually all the nitrogen will be washed out. The expression used in the calculation is the difference of the two alveolar values, " $\text{alv } \bar{a} - \text{alv } \bar{p}$ ". The likely error in each is a negative one. If the errors are equal, they will cancel each other. However, since that in " $\text{alv } \bar{p}$ " is probably very slight, the expression " $\text{alv } \bar{a} - \text{alv } \bar{p}$ " may be too small. Since this expression is the denominator of a fraction in the calculation, the final value for functional residual air by this method could be somewhat too large.

To take an example, an alveolar specimen in a case of emphysema might give a value of 82 per cent of nitrogen, when the average of all residual air nitrogen is actually 84 per cent. At the end of the period of oxygen breathing, let us say that the mean nitrogen of the residual air is 6 per cent. If the alveolar specimen as measured gives 4 per cent of nitrogen, then the alveolar *difference* ($0.82 - 0.04$) will be the same as the true difference of nitrogen concentrations in the residual air ($0.84 - 0.06$), the two errors thus cancelling. It is difficult to see how an error of method larger than 5 per cent would be likely from this source.

An attempt has been made to test for the presence of such an error. This test, analogous to that used for the Christie method in a previous paper, consists of a similar type of experiment in which the expired gases are collected during a period of breathing room air immediately following prolonged oxygen breathing. In other words, first all the nitrogen is washed out of the lungs, then, during a subsequent period of room air breathing, the amount of retained nitrogen in the lungs is measured.

Such a procedure is really a reversal of the oxygen breathing experiment, in so far as the direction of the nitrogen shift is concerned. In

this case, errors in alveolar measurement will cause an effect of opposite sign in the value obtained, as can be seen from analysis of the factors involved. Here the expression " $\text{alv } \bar{p} - \text{alv } \bar{a}$ " is the denominator of a fraction in the calculation. $\text{Alv } \bar{a}$, measuring the small nitrogen concentration after oxygen breathing, may possibly neglect nitrogen still trapped in the poorly aerated regions. Thus it is too low, if at all wrong. Similarly, $\text{alv } \bar{p}$, taken after a subsequent period of room air breathing, may be too high in nitrogen, since there may be some higher oxygen mixture still in the poorly aerated lung spaces. Thus the difference, " $\text{alv } \bar{p} - \text{alv } \bar{a}$," may be definitely too high, and from this, the residual air value too low. In contrast to the oxygen breathing experiment, from predictions in this case a cancellation of errors in alveolar measurements will be unlikely. Therefore it may be expected that this second open circuit method will be more likely to give erroneous results than the first method.

The test of the open circuit procedures will be a comparison of residual air values obtained by the two methods. If they differ, it will be evidence that the factor of poor mixing, as it influences alveolar samples, is still a possible source of error in the measurements. If they agree, one may consider the value obtained probably not significantly affected by erroneous alveolar measurements. It will be of special interest to compare this evidence with that obtained in the same subjects by the analogous test procedure in the closed circuit, reported in a previous paper.

PROCEDURE²

The apparatus for the open circuit methods was the same as that used for nitrogen excretion measurements in the first paper of this series. The diagram is reproduced here. The arrangement consists essentially of two open breathing circuits fitted with flutter valves (F_1, F_2, F_3, F_4) connected adjacent to the mouthpiece (M) at the valve (V_1), which can be used to shift the breathing from one circuit to the other. One circuit, the main circuit, is attached on its inspiratory side to a rubber anesthesia bag (B_1), this in turn to an oxygen tank. The expiratory side

²In collaboration with Dr Eleanor D Baldwin, we have recently devised a simplification of this open circuit method, based on results in 200 cases studied. In the new technique, alveolar sampling is eliminated, the only gas analysis necessary being that from the spirometer. This will be reported in a subsequent paper.

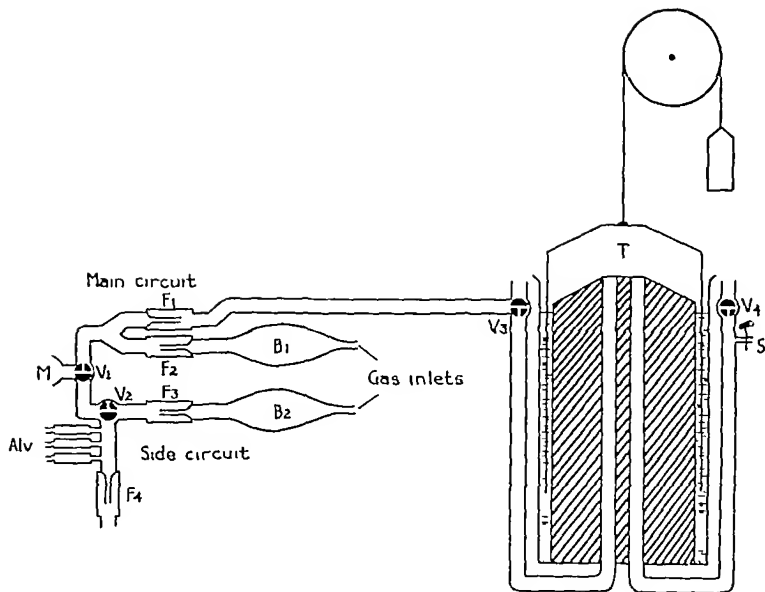


FIG. 1 OPEN CIRCUIT APPARATUS FOR MEASUREMENT OF RESIDUAL AIR

M mouthpiece. *Alv*, group of three evacuated gas sampling tubes. V_1 , V_2 , V_3 , V_4 , three-way respiratory valves. F_1 , F_2 , F_3 , F_4 , one-way rubber flutter valves. B_1 , B_2 , small rubber anesthesia bags. *T*, one-hundred liter (Tiasot) gasometer. *S*, valve and attachment for obtaining gasometer samples. For further explanation see text.

leads to a Tissot gasometer of 100-liter capacity. On the side circuit there is an additional valve (V_2) with which the inspiratory gas flow can be cut off during alveolar sampling. The inspiratory arm of this circuit leads to an anesthesia bag (B_2) and oxygen tank, which were replaced by a tube leading from outside air when atmospheric air was the desired breathing mixture. On the expiratory arm evacuated sampling tubes labelled *alv* are inserted close to the valve (V_1). The dead space from mouthpiece to these tubes is about 100 cc.

The procedure for a determination of functional residual air by the open circuit method was started with V_1 turned to the side circuit. The main circuit and gasometer were thoroughly washed out with oxygen. Six successive washings of 10 to 20 liters each were found adequate. After the washing V_2 was opened to connect the main circuit to the open room and a flow of 4 to 5 liters per minute of oxygen maintained in this circuit. The bag (B_1) in the side circuit was replaced by a room inlet tube. Then with V_1 unchanged the subject under basal conditions, was attached to the mouthpiece. When breathing quietly he was instructed to exhale maximally for an alveolar sample. At the same time the valve (V_1) was turned to close the

inspiratory side of the circuit. The alveolar sample was taken at the end of approximately five seconds of expiration and V_2 then reopened. This sample, designated *alv*, was thus a Haldane-Priestley alveolar sample and represented an attempted measure of average lung gas concentration on room air breathing.

Following this sampling at least two minutes of room air breathing were allowed in order to restore quiet breathing. Then V_1 was turned to direct the oxygen flow of the main circuit into the gasometer and V_2 was turned to the main circuit at exactly the end of a normal expiration. By watching carefully the respiratory rhythm for the few previous breaths, this latter valve turn could be made accurately at the desired moment.

For the next seven minutes of oxygen breathing no expired gases were collected in the gasometer. During this time, the oxygen flow was maintained to keep the bag (B_1) about one half full. The period of seven minutes was the standard one used. Results of trials were not presented to show that seven minutes was probably adequate.

At the conclusion of the seven minutes the valve V_1 was again turned to the side circuit, this time at the end

during expiration, preferably near the beginning. At the same time, the subject was instructed to expire fully for an alveolar sample. For this, as for all alveolar sampling, the valve (V_2) had been turned to close the inspiratory arm of the side circuit. This alveolar sample, designated "alv \bar{p} ," was taken at approximately five seconds of expiration as before.

Following this, the patient was disconnected and the main circuit flushed out with 5 to 10 liters of oxygen, the wash gas being allowed to mix in the gasometer with the collected expired gases. The valve (V_1) was next turned to close the entire gasometer contents, whose volume and the temperature were then recorded. A sample was taken from the gasometer for analysis within one to two minutes, after first flushing out the inlet and outlet pipes of the gasometer proper with the collected gases. This sample will be designated as "Tissot" sample in future references.

An approximation of the dead space of the gasometer was necessary for the final calculation. It will become apparent from the calculation to be discussed that this need only be an approximation. With the procedure as outlined, the effective dead space consisted only of the gas space under the bell when fully lowered. The dimensions of this space were measured and its volume calculated geometrically. The volume of the tubing did not need to be considered, since it was filled with oxygen at both the start and the end of the experiment.

Each experiment required the analysis of three gas samples, two "alveolar" and one "Tissot." In addition, the contents of each new oxygen tank required analysis for the small amount of inert gases. All gases were analyzed with a Haldane gas analysis apparatus. In the case of the samples of very high oxygen content, the dilution method was employed, as described in the first paper of this series. (The Van Slyke-Neill manometric apparatus can also be used for gas analysis.) Analysis of the alveolar specimens required an accuracy of only 0.1 to 0.2 per cent, so that possibly a simpler method might be employed. However, a high degree of accuracy was necessary in the analysis of the Tissot sample. In all cases the analyses were done in duplicate to check within 0.05 per cent. All analyses were reported as decimal fractions of an atmosphere of nitrogen, as dry gas.

CALCULATION

The first step in the calculation requires an expression for the total nitrogen in the expired gas in excess over that inspired. The volume of expired gas is known, but that inspired unknown. However, the nitrogen content of the inspired gas is so low that no significant error is involved by assuming that the inspired volume equals the expired volume. The gasometer volume obtained and corrected to dry gas at standard temperature and barometric pressure will be designated as V_0 . To this the measured dead space volume ($D S$) has been added for the calculation.

Then (1) Total excess N_2 in gasometer

$$= (V_0 + D S) ("Tissot" N_2 - "O_2 \text{ tank}" N_2)$$

Of this, a part has come from nitrogen originally in the lung spaces, a further part from excreted nitrogen from

the blood

$$(2) N_2 \text{ from functional residual air (F R A)} \\ = (V_0 + D S) ("Tissot" N_2 - "O_2 \text{ tank}" N_2) - N_2 \text{ excreted} \\ \text{Also (3) } N_2 \text{ from functional residual air}$$

$$= \text{F R A. Vol (alv } \bar{a} - \text{alv } \bar{p}) \\ (4) \text{ F R A. Vol} \\ = \frac{(V_0 + D S) ("Tissot" N_2 - "O_2 \text{ tank}" N_2) - N_2 \text{ excreted}}{\text{alv } \bar{a} - \text{alv } \bar{p}}$$

From the first paper of this series

$$(5) N_2 \text{ excreted} = 220 \times \frac{\text{alv } \bar{a} - \text{alv } \bar{p}}{0.80}$$

(For seven minutes, 10 cc added to 220 cc for each minute after seven, if longer period.)

Substituting

$$(6) \text{ F R A} \\ = \frac{(V_0 + D S) ("Tissot" N_2 - "O_2 \text{ tank}" N_2) - 275}{\text{alv } \bar{a} - \text{alv } \bar{p}}$$

The F R A value here obtained was then corrected to temperature 37° C and saturation with water vapor to obtain the values reported in our results.

The reversed procedure used to test these results was somewhat more complicated in practice but similar in principle. In this case the bag (B_1) was replaced with an inlet tube from outside air. The bag (B_2) was in place and connected with an oxygen tank. In preparation the main circuit and gasometer were thoroughly washed with room air. The subject was attached to the mouthpiece with valve V_1 open to the side circuit and an oxygen flow of 4 to 5 liters per minute in that circuit. Quiet breathing of oxygen was maintained for ten minutes. At the end of that time, an alveolar specimen was taken in the usual manner and quiet oxygen breathing was resumed in the same circuit for two further minutes. This alveolar specimen was designated as "alv \bar{a} " and considered as a measure of lung gas concentrations two minutes later, since it had been found by a series of tests that the alveolar nitrogen value during oxygen breathing reached a plateau value after ten minutes or less in both normal subjects and patients with emphysema, the maximum change in alveolar nitrogen from tenth to twelfth minute being less than 1 per cent.

At the end of the complete twelve minutes of oxygen breathing, the valve (V_1) was turned to the main circuit exactly at the end of a normal expiration. The expired gases were then collected for a seven-minute period (or longer) of room air breathing, after which an alveolar specimen was taken in the side circuit as before. The tubing of the main circuit was then flushed into the gasometer with 5 to 10 liters of room air, the valve (V_1) closed, and the gasometer volume and temperature read. A gasometer sample was taken promptly as before, after first flushing out the tubing of the gasometer itself with the first part of the collected gas.

As in the previous procedure, there were three gas samples and one volume measurement. In this case, however, the inspiratory volume, as well as the expiratory

volume, needed to be known for the calculation. Since the respiratory quotient was normally less than unity under basal conditions, the inspiratory volume was greater than the expiratory volume by an amount of some 200 to 400 cc. for the seven minutes. Direct measurement of the inspiratory volume with sufficient accuracy was found to be technically cumbersome. In our experience it was simpler to decide upon a correction factor (ΔV) to be added to the expiratory volume in order to determine the inspiratory volume. To do this the carbon dioxide excretion and the oxygen consumption per minute were determined from a six minute collection of expired air on room air breathing usually done just before the other tests on the same day. From this, (O_2 absorbed per minute - CO_2 excreted per minute) $\times 7 = \Delta V$ for seven minutes of room air breathing. This would seem to be an accurate correction in subjects with normal arterial oxygen saturation.

In cases with arterial oxygen unsaturation however there is an excess oxygen absorption during the first few minutes of oxygen breathing. Following resumption of room air breathing after oxygen there is a diminished oxygen intake until the arterial unsaturation is reestablished. It may be assumed with sufficient accuracy for the present purpose that the excess oxygen intake in one instance equals the diminution in the other. Accordingly in such cases the oxygen deficit of the subject was estimated from respiratory tracings taken with a recording spirometer. This oxygen deficit was subtracted from the ΔV value obtained above giving a corrected ΔV .

The calculation proceeded in an analogous manner to that in the first method. In this instance there was nitrogen retained in the lungs instead of washed out.

(1) N_2 retained in lung

$$= (V_e + D.S. + \Delta V) 0.791 - (V_e + D.S.) \text{ Tissot } N_2 - N_2 \text{ absorbed into blood}$$

Also (2) N_2 retained in lung = F.R.A. (alv p - alv d) and

(3) N_2 absorbed into blood = $220 \times \frac{\text{alv } p - \text{alv } d}{0.80}$

(4) F.R.A.

$$= \frac{(V_e + D.S.) (0.791 - \text{Tissot } N_2) + 0.791 \times \Delta V}{\text{alv } p - \text{alv } d} - 275$$

RESULTS

The subjects for this study were selected by the same criteria as those in the preceding paper. There were four normal subjects, including the two in whom the closed circuit method gave doubtful results. In all there were ten patients with severe pulmonary emphysema of whom six were included in the series of the previous paper. These six were studied by both of the new methods described. The remaining four were tested only by the first of the two new methods, which is the one proposed as a practical test.

It will be apparent, from the two preceding papers of this series as well as this present one

that this new method for residual air has been devised primarily for the study of cases of abnormal, unequal or ineffective pulmonary ventilation in which previous methods, such as that of Christie, have been found inaccurate. It is on the basis of such unusual or extreme cases that any new method must be judged. If in these cases or at least in a fair proportion of them, the method can provide a reasonably accurate and consistent measure of residual air, then the method can perhaps be considered worthy of further trial.

The results should be examined, therefore case by case, rather than by any attempt at statistical analysis.

The results of tests on these subjects may be compared in several ways.

(1) Comparison of results by each of the two open circuit techniques (Table I). This will demonstrate whether the slowness or poorness of distribution of respiratory gases, in any given case is sufficient to invalidate the technique used.

TABLE I

Functional residual air determinations by open circuit methods

NORMAL SUBJECTS						
Subject	Length of test	Number of determinations	Decreasing lung N_2		Increasing lung N_2	
			Average	Range	Average	Range
J.L.	minutes	8	1220	(1140-1300)	1060	(1005-1060)
A.C.		3	2043	(2016-2068)	1920	(1880-1980)
R.C.D.		3	2690	(2665-3007)	2770	(3410-3007)
D.W.B.		2	2456	(2250-2650)	2355	(2310-2400)
						Open circuit
						Closed circuit
						Ratio of average values*
						0.87
						1.02
						0.83
						0.91

SUBJECTS WITH PULMONARY EMPHYSEMA

M.E.		2	4065	(4030-4100)	3815	(3760-3950)	0.93	0.73
Am.C.		2	2224	(2060-2370)	2345	(2760-2250)	1.01	0.83
F.H.		1	2670		2340		0.96	0.84
J.C.	7	3	2690	(2640-2730)	2440	(2520-2740)	0.91	0.83
	10	1	2150		2785		0.92	0.87
D.H.	7	4	2235	(2050-2390)	2235	(2800-2910)	0.85	0.81
	10	1	2325		2390		0.97	
H.K.	7	4	2960	(2935-3140)	2935	(1700-2140)	0.71	0.56
	10	3	3090	(2855-3235)	2190	(2445-2120)	0.81	0.53

* Ratio = average value by increasing lung nitrogen method ÷ average value by decreasing lung nitrogen method.

† Figures computed from Paper II of this series.

(2) Comparison of the relative agreement between the two open circuit techniques, with the relative agreement between the two closed circuit techniques. This is given by the two ratios, in the last two columns of Table I.

(3) Average values by the open circuit method can be compared with average values in the same subject by the closed circuit method of Christie (Table III). This will provide, for any given case, a criterion as to whether the various sources of error in the Christie method do actually cancel out, leaving a residual air value comparable with that of the open circuit method. In this comparison it will be important to note not only the difference of average results, but also to compare the range or reproducibility of results by the two methods.

Table I presents the first type of comparison, listing the average open circuit results, the range, and number of determinations. In the two columns on the right are listed also the ratio of the two values by the open circuit methods parallel with the ratio of the two closed circuit results in the same subjects.

Among the four normal subjects, it will be seen that all agree within 200 cc. These include the two subjects, D W R and R C D, who showed 500 and 900 cc difference, respectively, with the two closed circuit methods. Thus it may be seen that in this group of normal subjects there is no evidence of serious error due to maldistribution in the open circuit methods.

It may be noted that the open circuit methods in the case of J L give somewhat poorer agreement than the closed circuit. The reason for this is not entirely clear. It seems probable, however, that the error lies in the increasing lung nitrogen method. In the case of small subjects, the assumed ΔV factor in this method has a much larger relative influence on the result than in larger subjects.

Considering now the six abnormal subjects, it will be seen that the first four show satisfactory agreement between the two methods. Agreement within 10 per cent may be considered satisfactory. The fifth subject, D H, showed a 13 per cent difference by the standard seven-minute test. Using a twelve-minute period, however, there was good agreement. It should be noted

that the only significant change by the twelve-minute test was an increase in the result by the second method. This is a part of the evidence which leads to the tentative conclusion that a seven-minute period is adequate for the decreasing lung nitrogen method.

The sixth subject is the only one in whom agreement could not be reached. This case will be discussed in detail later.

The striking difference between the open and closed circuit methods, tested by analogous procedures, is shown in the last two columns, which may be considered as comparative indices of the influence of poor mixing on the results. It will be seen that, in every instance among the abnormal subjects, the index shows much better agreement by the two open circuit techniques.

Table II presents in detail the results on the subject H K, the sixth one in Table I, in whom

TABLE II
Functional residual air by open circuit methods
Subject—H K

Date	Decreasing lung N ₂ method			Increasing lung N ₂ method		
December 15 1938	2895			2385		
December 16 1938	3095			1700		
December 20 1938	3140			2560		
December 24 1938	3110*			2510*		
December 27 1938	3285†			2520†		
January 17 1939	2700			1735		
	2855†			2445†		
		S D	S E _m		S D	S E _m
Mean 7 minute value	2960	130	65	2095	510	255
Mean 11 minute-12 minute value	3080	275	160	2490	50	29

* 11 minutes

† 12 minutes

agreement could not be reached. This subject was a man of sixty-five with a history of increasing cough and dyspnea for twelve years. The etiology of his condition was unknown. There was no evidence of tuberculosis. There were diffuse asthmatic rales, but no improvement with the use of adrenalin. Roentgenogram of the chest showed increased hilar markings and dark areas at the periphery suggesting emphysematous bullae. His arterial oxygen saturation was 85 per cent. At the time of testing, he was practically bedridden because of dyspnea, though this symptom varied considerably from day to day.

As shown in Table II, there was a considerable variation in values obtained for functional re-

residual air in this subject in experiments continued over a month & time. It will be noted, however, that the figures by the decreasing lung nitrogen technique are rather consistent considering that the subject's clinical state varied considerably from one day to the next. It was the increasing lung nitrogen technique which gave the wider variations, and these figures generally are lower than the other group. This is the type of error which is to be expected (see above) with the increasing nitrogen technique when intrapulmonary mixture is extremely poor. Furthermore by prolonging the period of breathing such an error should become less, and Table II in the second column, shows a clear tendency for the figures in the eleven- and twelve minute period to be higher than the seven minute period. In the first column, little difference is noted with prolongation of the breathing period.

Table III presents the data for comparison of open and closed circuit techniques in the same

TABLE III

Comparison of functional residual air values by Christie modified Christie and new open circuit methods

NORMAL SUBJECTS							
Sub- ject	Closed circuit			Open circuit			Ratio of Mean Values
	Modified Christie method			Decreasing lung N ₂			
	Num ber	Mean	Range	Num ber	Mean	Range	
J.L.	12	1320	(1170-1485)	5	1220	(1140-1300)	1.08
A.C.	2	2235	(2135-2315)	2	2180	(2100-2265)	1.02
D.W.R.	3	3540	(3200-3770)	2	3450	(3250-3650)	1.02
R.C.D.	5	3250	(2890-3545)	3	2890	(2695-3080)	1.13
SUBJECTS WITH PULMONARY EMPHYSEMA							
Ant.C.	2	3240	(3140-3340)	2	3225	(3080-3370)	1.00
A.M.	6	2750	(2790-3435)	4	2570	(2550-2600)	1.07
A.H.	2	3235	(3140-3270)	2	3590	(3580-3600)	0.91
D.H.	2	3140	(3070-3210)	5	3355	(3050-3590)	0.94
J.C.	4	6570	(5980-7760)	4	6650	(5940-6250)	1.08
V.L.	2	3630	(3615-3645)	1	3515		1.03
V.H.	1	3020		1	2670		1.13
H.K.	3	3835	(3560-4060)	7	3010	(2855-3285)	1.27
M.S.	6		(2230-3750)	8		(2420-3570)	
M.K.	10		(2245-3260)	12		(2130-4100)	

subjects, listing the results by the standard Christie procedure, calculated with alveolar specimens, and by the open circuit method using the decreasing lung nitrogen technique.

Among the normal subjects the first two gave results in close agreement by the modified Christie and the new methods as expected from the fact that previous analysis indicated both

methods were reliable. In the case of the third subject, D.W.R., with whom the closed circuit could not be proved reliable still the two circuits gave practically the same results. It is apparent here that in the standard closed circuit method there must have been a fair balance of the various errors which were mentioned.

In the case of the fourth subject, R.C.D. in whom also the closed circuit gave doubtful results, the open circuit gave significantly lower results with less variability on successive tests and with a good agreement with both open circuit techniques. Thus it would seem here that the standard closed circuit method gave an erroneously high value.

The group of abnormal subjects includes ten patients. The last two of these were unusual cases however, and will be presented in detail in a later tabulation.

Considering for the moment the first eight subjects only as far as a comparison of the average results, it will be seen that the first six show no significant differences between average open and closed circuit results. As before a difference of less than 10 per cent is considered insignificant. In the seventh case F.H. and more markedly in the eighth case H.K. the closed circuit values are significantly higher.

It is probably important that these two subjects had the greatest pulmonary disability of the group.

Let us next note the reproducibility of results by the two methods in these same eight patients. In two of them A.M. and J.C., the open circuit shows much less difference in successive tests. The other subjects either showed a similar range or else the data are insufficient to determine the range. It should be mentioned that the range of values in many of the subjects probably reflects not only the accuracy of the method, but also the actual volume variation from day to day.

Table IV presents the detailed results on the two remaining subjects M.S. and M.K. They are considered together because of their similar clinical pictures and comparable results on residual air determinations. They were both men of slightly under forty years of age, with prolonged histories of nearly constant asthma and bronchitis. Each had marked arterial anoxemia and secondary polycythemia. M.K. had had

TABLE IV
Functional residual air

Date	Closed circuit Modified Christie method	Open circuit Decreasing lung N ₂
Subject—M S		
October 19	2285	
	3295	
October 24		2420
		3110
October 30		3570
		3220
		2815
		3070
November 8	2590	
	2230	
November 18*	3750	3140
	3310	3210
December 26*	2450	2850
	2805	2410
Subject—M K		
May 7 1938	3890	
May 13, 1938	3550	
May 17, 1938	2710	
	3125	
September 21, 1938		4030
September 22, 1938		4100
October 19, 1939	4250	
	5260	
October 21, 1939		3230
		2780
October 26, 1939		2240
		2130
		3050
October 28, 1939		3605
		4050
		3450
November 15 1939*	2530	2920
	2245	2760
December 28, 1939*	3220	2975
	2955	2660

* Tests on these dates performed after the use of "Vaponefrin" bronchodilator spray

several bouts of right-sided cardiac decompensation with massive edema, but was compensated at the time of these measurements. M S had slight cardiac weakness only, and had had one episode of mild edema. Each of these patients had a large element of bronchial spasm in his disability, as shown by partial relief with adrenalin. However, free of asthma with adrenalin, they still had arterial anoxemia.

A striking feature in both these subjects was the extreme variability of their respiratory disability, and likewise their extremely irregular respiratory curves. In the case of M K, on one occasion following a cough, a sudden shift of 1000 cc was noted in the base line of a respiratory tracing. This variability of actual residual air volume would seem to be indicated in the measurements taken up to the last two days of tests

in each case. Whether either method is also in error cannot be deduced from such variable data.

In order to obtain a more stable condition, each of these subjects was tested in duplicate by both methods, following the use of "Vaponefrin" spray inhalation, or adrenalin by hypodermic injection. The results of these tests are listed on the last two days in each subject. Very little more can be said from these results except that the variability from test to test on the same day seems to be reduced. It is obvious that average values in these two subjects have no meaning. One can only conclude that these subjects are unsuitable for residual air measurements by either the closed or open circuits. Certainly a part of this difficulty is due to actual change in the functional residual air volume itself. It may be that poor intrapulmonary gas mixture is a factor, producing significant errors of measurement. Their extremely slow mixture is shown by their values for alv \bar{p} nitrogen, which were frequently as high as 10 per cent after seven minutes of oxygen breathing.

DISCUSSION

It is apparent from the results that the new method does not entirely solve the problem of accurate residual air measurement. It has been shown and should be again emphasized that, in severely diseased lungs and probably also in some normal individuals, there is an imperfect mixture of gases within the lungs during breathing. This factor may be an important one in any method involving the estimation of average gas concentrations within the lungs. Since no method without such estimations was found, the aim of the new method was to minimize the effect of these potentially erroneous measurements.

The theoretical advantages of the new method were clear cut. By keeping the inspiratory gas mixture of constant composition, it offered a better chance of adequate intrapulmonary mixing. The "mixing" approached by the procedure is actually a simple process of *emptying* the lung of all, or nearly all, its contained nitrogen. By increasing the net alveolar nitrogen change, the new method reduced the relative error due to erroneous "alveolar" samples. Furthermore, it

avoided the necessity of measuring oxygen consumption from a respiratory tracing. Accuracy in this is necessary in the Christie method, yet often difficult in patients with emphysema or other pulmonary conditions.

The new method, tested on a small group of picked subjects, has shown definite improvement over the closed circuit method, in so far as the probable effect of poor mixing is concerned. The method of testing this effect has been the same as that used for the closed circuit, namely, the repetition of the test with a reversal in the direction of the nitrogen shift by which the probable error due to poor mixing was also reversed in direction. On the basis of such a test, the new method showed no evidence of error in any normal subjects nor in any but one of the patients with pulmonary disease. Even in this one subject, the trend of repeated tests increasing the breathing time indicated the probable accurate figure. This was a contrast to similar evidence by the closed circuit method where two normal subjects and all subjects with emphysema showed considerable discrepancies due to poor mixing.

With this evidence in its favor, the results by the open circuit method have been compared with the results obtained in the same subjects by the closed circuit. Three normal subjects and six patients with emphysema gave nearly identical values by open and closed circuit methods respectively. One normal subject and two patients with severe emphysema gave significantly higher results by the closed circuit technique. This is in accordance with the prediction offered in the analysis of possible errors of method in the second paper of this series. In addition, two other patients with emphysema showed a considerably wider range of variation in residual air values on successive tests by the closed as compared with the open circuit method.

It is thus apparent that the new method can be used with some assurance of reliability in a larger group of abnormal subjects in whom such measurements are notoriously difficult. It would seem that this point is its chief practical justification, in addition to the theoretical advantages mentioned.

Even in our small group of subjects however, there were two unusual cases in whom both

methods gave variable results. A part of this was undoubtedly due to actual change in the volume, but still it leaves two exceptions in whom the reliability of the method could not be proved. In such cases it would be desirable to have a method in which no problem of mixing is involved.

Our primary interest has been the identification and quantitation of disturbances in mixing. Both an increase in the residual air and a disturbance in mixing may add to the pulmonary disability in cases of emphysema. In cases where the maldistribution factor is large, the closed circuit method may give a value which represents the true functional residual volume plus an increment that is actually an error of method due to poor mixing of intrapulmonary gases. This factor of disturbed distribution is probably a large one in contributing to the pulmonary disability in emphysema. Therefore it is important to differentiate it from the effect of a mere increase in the residual lung volume. The open circuit method should give a better measure of the latter without serious error from the former. With an accurate measure of the volume it should be possible to quantitate disturbances in distribution separately.

SUMMARY

1 A new open circuit method of measuring residual air is described.

2 A test for this method is also described consisting of a reversal of the nitrogen shift in the original procedure.

3 Results of the use of these two procedures are presented on a group of four normal subjects and six patients with severe pulmonary emphysema.

4 A comparison is made between results by the new method and by the modified Christie method on a series of four normal subjects and ten patients with severe pulmonary emphysema.

5 The probable significance of the results is discussed.

CONCLUSIONS

1 The open circuit method for residual air determination offers a better means of avoiding

error due to maldistribution of pulmonary gases than the closed circuit method. This is shown by the test procedure as well as theoretical considerations.

2 The modified Christie method gives erroneously high values for residual air in some cases of severe pulmonary emphysema.

3 It is possible with the new method to distinguish more surely the factor of imperfect gas distribution from that of excessive residual lung volume in the evaluation of the disturbances associated with pulmonary emphysema.

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STUDIES ON ANACIDITY THE HYDROGEN-ION CONCENTRATION OF THE GASTRIC SECRETION THE GASTROSCOPIC APPEARANCE OF THE GASTRIC MUCOSA AND THE PRESENCE OF A GASTRIC SECRETORY DEPRESSANT IN PATIENTS WITH ANACIDITY

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Clinicians have customarily used the indicator, dimethyl amino-azobenzol (Toepfer's solution) to indicate the presence or absence of free hydrochloric acid in the gastric juice. Bloomfield and Pollard (1) have pointed out that in many such instances of apparent anacidity with this indicator, the stomach is still capable of secreting hydrochloric acid and that by determining the hydrogen ion concentration, the pH may be found definitely on the acid side of neutrality. Further more in these cases there is no regular response to histamine and such secretions as one obtains do not conform to the normal curves but seem to represent a minimal continuous secretion largely or completely independent of the stimuli which normally cause a flow of juice." In this regard Martin (2) found a wide variation in the chloride content of the gastric secretion from patients with so-called 'benign achlorhydria'. The concentration of ammonia and non protein nitrogen was considerably greater than that found in the acid-secreting patients. This increase was still more pronounced in pernicious anemia and gastric carcinoma with achlorhydria. After histamine stimulation there was usually some change in the acid base pattern but not to any significant degree.

Many people, without apparent disease, may have a permanent anacidity. Vanzant *et al* (3) noted an incidence of 14.5 per cent after the use of a modified fractional meal. Pollard (4) reported an incidence of 10.8 per cent true anacidity in 900 cases (excluding pernicious anemia and carcinoma of the stomach). Furthermore an acidity increases in frequency with advancing age (3). The relation of gastric anacidity to disease has been considered in detail in the excellent monograph of Bloomfield and Pollard (1). An acidity is an invariable accompaniment of pernicious anemia and occurs frequently in carcinoma of the stomach. Schundler *et al* (5) in describing the anatomic foundations of anacidity include 6

cases of hypertrophic gastritis, 12 of superficial gastritis and 37 of atrophic gastritis in a series of 120 cases. Brunschwig *et al* (6) have recently reported that when the gastric secretions from 16 of 18 patients with pernicious anemia (89 per cent) were injected intravenously in dogs with stimulated gastric pouches, they produced a marked inhibition of pouch secretion and achlorhydria. Only 18 per cent of a control group produced similar effects.

The following investigation was undertaken to determine whether significant differences in the anacidity associated with these various conditions would be revealed by a study of the hydrogen ion concentration of the gastric secretion and whether or not this could be correlated with the gastroscopic appearance of the gastric mucosa or the presence in the gastric juice of a secretory depressant. For this purpose the pH of individual samples of the gastric secretion obtained after histamine stimulation was determined electrometrically with a Beckman pH meter. The standard histamine test was employed. In every instance the position of the tip of the Rehfuess tube was checked fluoroscopically. The patients were cautioned to avoid swallowing saliva. After the fasting contents were withdrawn histamine ('mido' Roche) was injected hypodermically in doses of 0.01 mgm per kilogram of body weight. The stomach contents were then aspirated at 10-minute intervals for one hour. The data comprise pH determinations in 854 specimens of gastric secretion obtained in a series of 72 patients (44 men and 28 women). In 45 patients the absence of free hydrochloric acid (as denoted by the presence of a yellow color with Toepfer's solution) was confirmed by more than one histamine test. The presence or absence of a secretory depressant was determined by Dr Brunschwig in 19 patients.

Pernicious anemia

The 17 patients with pernicious anemia include 8 men and 9 women. The ages ranged from 29 to 69. All except 2 cases (J C and F L) had received liver therapy. The fasting pH varied from 6.70 to 8.50. After histamine stimulation there was no significant lowering of the pH, the figures ranging from 6.40 to 8.81 (Figure 1). In all but 4 cases the fasting pH was consistently lower than the pH after stimulation. The average values were 7.43, 7.81, 7.87, 7.95, 8.04, 8.01, and 7.99. In one of the 8 patients gastroscopied (F M) a normal stomach was observed. The changes in the other cases varied from a patchy atrophic gastritis to extensive atrophy. There was no direct relationship between the severity of the gastroscopic picture, the presence or absence of anemia, and the hydrogen-ion concentration. Helmer, Fouts, and Zerfas (7) found that the pH in 47 patients with pernicious anemia varied from

6.9 to 8.6. In 14, a measurable drop in pH occurred after histamine stimulation. Streicher *et al* (8) observed a pH range of 6.5 to 7.2 in 4 cases of pernicious anemia. Six patients of the present series were found to possess a gastric secretory depressant, but there was no correlation between these findings and the hydrogen-ion concentration of the respective gastric secretions.

Atrophic gastritis

In this group are included 19 patients (10 men and 9 women), the ages ranged from 28 to 68. The blood counts in all cases were within normal limits. The fasting pH varied from 6.51 to 8.60. After histamine stimulation, a definite drop in pH occurred in 8 cases. The figures ranged from 4.52 to 8.59 (Figure 2). The average values were 7.47, 7.74, 7.60, 7.00, 6.97, 7.09, and 6.90. Although the pH curve resembles that of pernicious anemia, the range is distinctly greater. It is

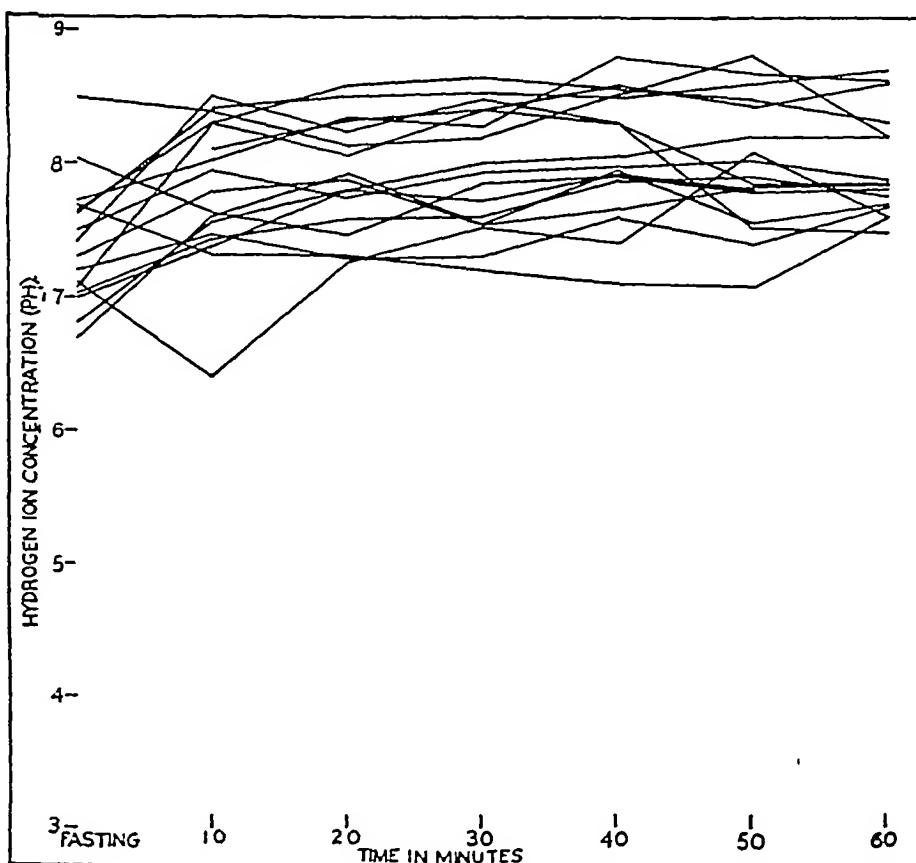


FIG. 1 INDIVIDUAL pH VALUES IN PERNICIOUS ANEMIA (HISTAMINE STIMULATION)

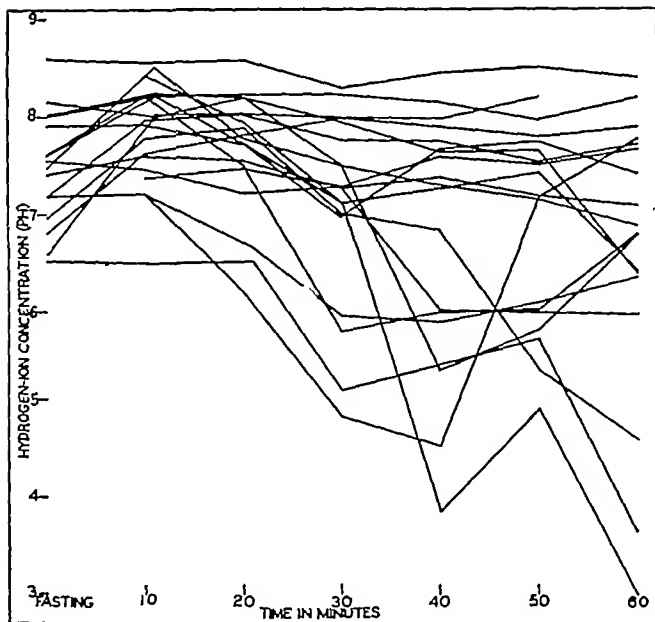


FIG. 2. INDIVIDUAL pH VALUES IN ATROPHIC GASTRITIS WITH ANACIDITY (HISTAMINE STIMULATION)

interesting to note in this connection that Schindler and Serby (9) were unable to differentiate gastroscopically between the atrophic gastritis seen in pernicious anemia and that seen in other types of anemia or in cases without anemia. The extent and severity of the atrophic gastritis in this group could not be correlated with the hydrogen ion concentration of the gastric secretions. Five patients were tested for the secretory depressant of Brunschwig, positive results were obtained in 3 and negative results in 2. These observations were related neither to the pH nor to the degree of inflammation. Some patients had been treated with liver extract and ventriculin without significant change in the pH values after such therapy. The case of J.M. is interesting in that it represents an instance of subacute combined cord degeneration without anemia and with anacidity. Palmer and Porter (10) have described this patient in detail previously. Oliver and Wilkinson

(11) have observed an incidence of 100 per cent achylia gastrica in 39 such patients.

Carcinoma of stomach

The 8 cases included in this group were all males with ages ranging between 50 and 70. Secondary anemia was present in 4 patients. The fasting pH varied from 6.68 to 8.50. After histamine stimulation, a definite drop in pH occurred in 4 cases the pH ranging from 4.81 to 8.54 (Figure 3). The average pH values were 7.46, 7.36, 6.99, 6.51, 6.45, 6.98 and 7.37. In all patients the carcinoma was extensive. Five patients were gastroscopied, in 2 there was an associated extensive atrophic gastritis, in 2 a patchy atrophic gastritis while in the fifth case there was no evidence of atrophy. Brunschwig *et al.* (12) continuing their important studies found that the gastric secretions from 21 of 27 patients with carcinoma of the stomach (78 per cent) contained

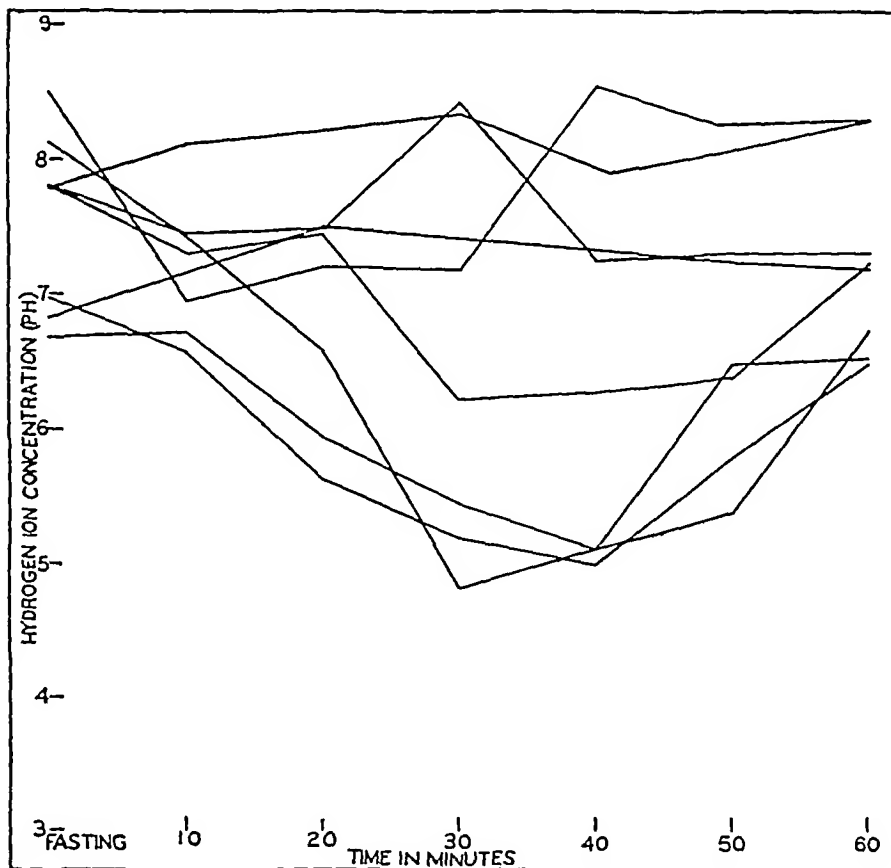


FIG 3 INDIVIDUAL pH VALUES IN GASTRIC CARCINOMA WITH ANACIDITY (HISTAMINE STIMULATION)

a secretory depressant. Of 3 patients in this group similarly studied, 2 yielded positive and one negative results. The pH could not be correlated with any of these observations.

Polland and Bloomfield (13) determined the hydrogen-ion concentration (colorimetrically) of the gastric juice in some cases of carcinoma of the stomach. When a complete absence of acid existed, the pH reading was usually 6 to 8. In several of the cases, even though no test for free acid was obtained with di-methyl, the pH fell in successive specimens after stimulation to a pH of 3 to 5, indicating that traces of acid were being secreted. These observations are in substantial agreement with the present data.

Anacidity after radiation therapy

Palmer and Templeton (14) recently have described the effects of radiation therapy on gastric secretion. The purpose of such treatment was

the production of anacidity artificially by x-ray therapy in ulcer patients, thus removing one of the causes essential to the formation of chronic gastroduodenal ulcer. Ten such cases with anacidity to Toepfer's solution are included in this study. All were men with ages ranging from 20 to 52. The amount of radiation therapy varied from 1055 R to 2930 R. The red blood counts and hemoglobin were uniformly normal. The fasting pH varied from 5.65 to 8.19. After histamine stimulation, there was a significant drop in the pH in 8 cases, the figures ranging from 4.13 to 8.00 (Figure 4). The average values were 7.17, 7.31, 6.89, 6.02, 5.79, 6.20, and 6.31. In 2 patients a superficial gastritis was seen, while in the third case a mild hypertrophic gastritis was observed. Negative results were obtained in 3 patients studied for the presence of a secretory depressant. The variations in pH after histamine stimulation were most pronounced in this group.

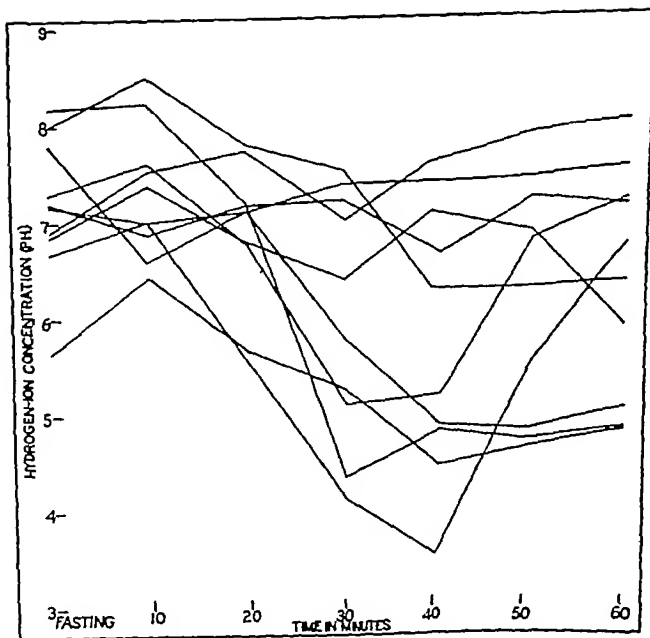


FIG. 4 INDIVIDUAL pH VALUES IN POST-RADIATION ANACIDITY
(HISTAMINE STIMULATION)

Miscellaneous conditions

Eighteen patients with various miscellaneous conditions were studied. In 3 cases all males, an extensive superficial gastritis was present. The secretion of one of these (J. E.) was found to contain the secretory depressant of Brunschwig. Cholelithiasis was found in 3 cases. Eight patients (3 men, 5 women) were diagnosed as functional bowel distress after complete roentgen studies of the gastro-intestinal tract were interpreted as normal. In 2 of these, a definite lowering of the pH occurred after histamine stimulation. The pH of the gastric secretions in some instances was similar to the values obtained in pernicious anemia or atrophic gastritis. Since none of these patients (excluding those diagnosed as superficial gastritis) were gastroscopied, the possible presence of an atrophic gastritis is not excluded.

DISCUSSION

The data presented in this study indicate that anacidity is most complete and constant in pernicious anemia. The pH varied between 7.0 and 8.0 with only slight variations after histamine stimulation. The pH in atrophic gastritis resembled that of pernicious anemia but was more variable. It is interesting to note that not only are the pH curves similar but also that the two conditions cannot be distinguished gastroscopically. In addition, although the series is small, a majority of the patients tested from these two groups were found to possess a secretory depressant in the gastric secretions. The pH values in carcinoma of the stomach and radiation therapy were distinctly lower than in the two preceding groups. These differences are well shown in the individual graphs and the average curves (Figure 5).

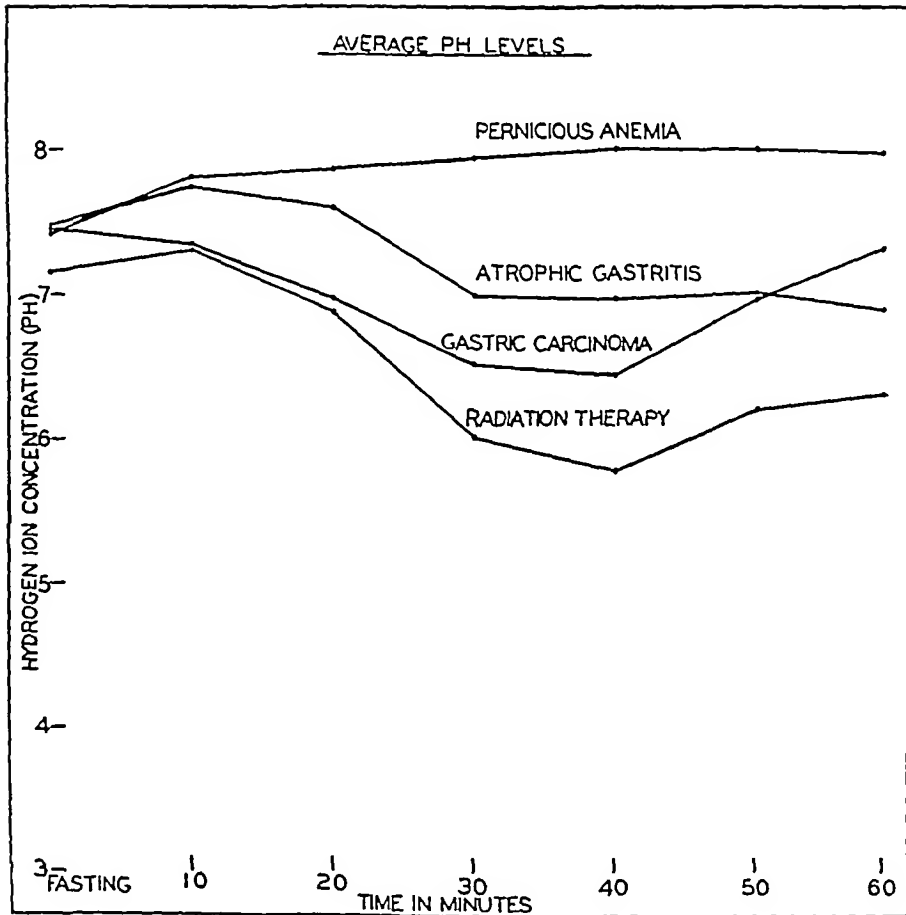


FIG 5 THE pH OF THE GASTRIC SECRETION IN ANACIDITY AVERAGE CURVES IN PERNICIOUS ANEMIA, ATROPHIC GASTRITIS, GASTRIC CARCINOMA AND POST-RADIATION ANACIDITY (HISTAMINE STIMULATION)

The pH curves in the anacidity associated with superficial gastritis, cholelithiasis or functional bowel distress may resemble those obtained for pernicious anemia, atrophic gastritis or carcinoma of the stomach, indicating no specific values for any particular type. The artificial anacidity which follows radiation therapy obviously is not as complete as in the other groups. Palmer and Templeton have shown, furthermore, that such an anacidity is not permanent.

No conclusions can be drawn from this study as to the mechanisms involved in the pathogenesis of spontaneous histamine-proved anacidity. The pH levels cannot be correlated with the gastroscopic picture, the presence or absence of a secretory depressant in the gastric secretions, or the presence or absence of anemia. Pollard (15)

likewise attaches no significance to anemia as a cause of anacidity.

SUMMARY AND CONCLUSIONS

The hydrogen-ion concentration of the gastric secretion obtained after histamine stimulation and showing a yellow color ("anacidity") to Toeper's reagent was determined in 72 patients (44 males, 28 females). The series comprised 17 patients with pernicious anemia, 19 patients with atrophic gastritis, 8 patients with carcinoma of the stomach, 10 patients with anacidity after radiation therapy, and 18 patients with miscellaneous conditions.

The pH of the gastric secretion in pernicious anemia falls within a range of pH 7.0 to 8.0 and shows no appreciable drop after histamine stimu-

lation The pH of the gastric secretion in atrophic gastritis resembles that of pernicious anemia but varies more than pernicious anemia after histamine stimulation.

The pH of the gastric secretion after histamine stimulation in carcinoma of the stomach with an acidity and in the anacidity which sometimes follows radiation therapy is lower and more variable than that found in pernicious anemia.

The individual pH curves in these groups may resemble one another thus suggesting that there is no specific pH curve for the anacidity associated with a particular disease process

There is no correlation between the hydrogen-ion concentration of the gastric secretion the gastroscopic appearance of the stomach mucosa, the presence or absence of a secretory depressant factor in the gastric secretion and the presence or absence of anemia.

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THE EFFECT OF EXERCISE ON THE VOLUME OF THE BLOOD

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The purpose of this investigation is to compare measurements of the blood volume and related factors in normal persons and in cases of heart disease before and after exercise. Previous in direct studies of the blood volume have indicated that as a result of exercise there is an increase in the concentration of the red blood cells and hemoglobin. Two explanations have been proposed to account for this change, (a) the red cells are extruded from the blood depots into the circulation thereby increasing the volume of the blood and cells (1, 2, 3) and (b) there is a change in the concentration of the blood due to passage of fluid from the blood into the tissues (4, 5).

The point at issue here should be readily clarified by direct measurements of the blood volume during and after exercise but the results of such determinations do not settle the problem because there is no unanimity of opinion. In dogs after short periods of exercise one group of observers (6) found a decrease in the plasma and cell volumes, while another observer (7) noted an increase in all the components of the blood volume. In man, Chang and Harrop (8), employing the carbon monoxide method for the blood volume, exercised 4 normal individuals on a bicycle ergometer for from 6 to 10 minutes and found that the total circulating blood volume was increased from 1.3 to 7.3 per cent. Ewig and Hinsberg (9) and Wollheim (10) using the dye method found an average increase of 11.8 per cent in the plasma volume and 15.1 per cent in the cell volume after short periods of moderate exertion in normal subjects. Levin (11) determined the plasma volume immediately after exercise in 11 subjects and found it decreased in 8 subjects, unchanged in 1 and increased in 2 while the cell volume was diminished in 4, increased in 4 and unchanged in 3 cases. Lozoya (12), working at an altitude of 2249 meters, concluded from his observations that one-half hour of exercise (running and jumping) diminished the plasma volume 8.9 per cent, increased the cell volume 5.6 per cent, and decreased

the total blood volume 4.1 per cent. Gibson and Branch (13) stated that they observed as a result of exercise a prompt and considerable decrease in the blood volume.

Observations on the blood volume before and after exercise in patients with heart disease are relatively few. Ewig and Hinsberg (9) and Wollheim (10) reported that while in healthy individuals exercise increased the circulating blood volume through mobilization from the blood depots, in heart disease physical activity was not followed by such an effect the volume remaining essentially the same. As the patient with heart disease regained his cardiac reserve, the response of the blood volume to exercise was again normal, i.e., an increase in plasma and cell volumes. The results of Levin (11) on heart disease are somewhat at variance with those reported by the above observers. This investigator found that the circulating blood volume increased in 58 per cent of the patients with heart disease (compensated and decompensated) in contrast to normal individuals in whom the volume was diminished in 73 per cent of cases as a result of exercise.

METHODS AND PROCEDURES

The plasma volume was measured by the dye method described by Gibson and Evans (14). The plasma volume is measured by determining the dilution in the blood stream of a measured amount of an azo dye, T-1824 after intravenous injection. Changes occurring during the experimental procedures were followed by the short "indirect method" (14). By this method changes in the plasma volume in excess of ± 40 cc. and -90 cc. may be considered significant.¹ The total blood and cell volumes

¹ The assumption is made that the dye disappears from the blood stream at a linear rate and that the best line fitting the five initial points gives the rate. Actually the rate of disappearance of most substances from the blood stream is proportional to the amount present, i.e. it is an exponential curve. In this series of experiments, we were interested to know what was the magnitude of the errors which occurred, due both to the experimental variation and to the assumption that the disappearance is a straight line. In 2 cases observations on the concentra-

were calculated from the plasma volume and the hematocrit values. The hemoglobin was determined by the oxygen combining power method (15), the blood viscosity by the Hess Viscometer and the proteins by Howe's Kjeldahl method (16). The circulation time was measured by the intravenous injection of "decholin" (17) and the venous pressure by the direct method of Griffith, Chamberlain and Kitchell (18).

The procedure of the experiments was as follows. All the subjects were examined under basal conditions. On coming to the laboratory in the morning the subject

tion of the dye in the serum were made for 90 minutes after the beginning of the experiment without doing anything to modify the blood volume. (See control curve, C. D., at the top of Figure 1.) The mean observed per cent error was -0.82 with a standard deviation of ± 1.04 per cent. This means that, as far as one may argue from these 2 cases, 94 per cent of the observations will be between $+1.26$ and -2.90 per cent or $+38$ cc. and -87 cc. for an average plasma volume of 3000 cc.

rested on the seat of a bicycle ergometer for 45 minutes. During and for 10 minutes prior to the determination of the plasma volume the subject's arm was placed in a comfortable stand so adjusted that the antecubital vein was at the height of the angle of Louis. A vein in the antecubital fossa was punctured and blood was withdrawn for a dye free sample of serum. An accurately measured amount of dye was then injected. After rinsing the syringe and needle several times with blood the venous pressure was measured. Fifteen minutes (18 to 20 minutes in patients with heart disease) following the dye injection blood samples were taken at 5-minute intervals for 20 minutes. After the last sample was obtained the circulation time was determined.

When the sampling was completed, the subject was asked to ride a stationary bicycle for 10 minutes, the normal subjects performing on an average 400 kpm. per minute and the patients with heart disease 350 kpm. per minute. Samples of blood were withdrawn from the antecubital vein about half way through the exercise period and just before the exercise was terminated. Dur-

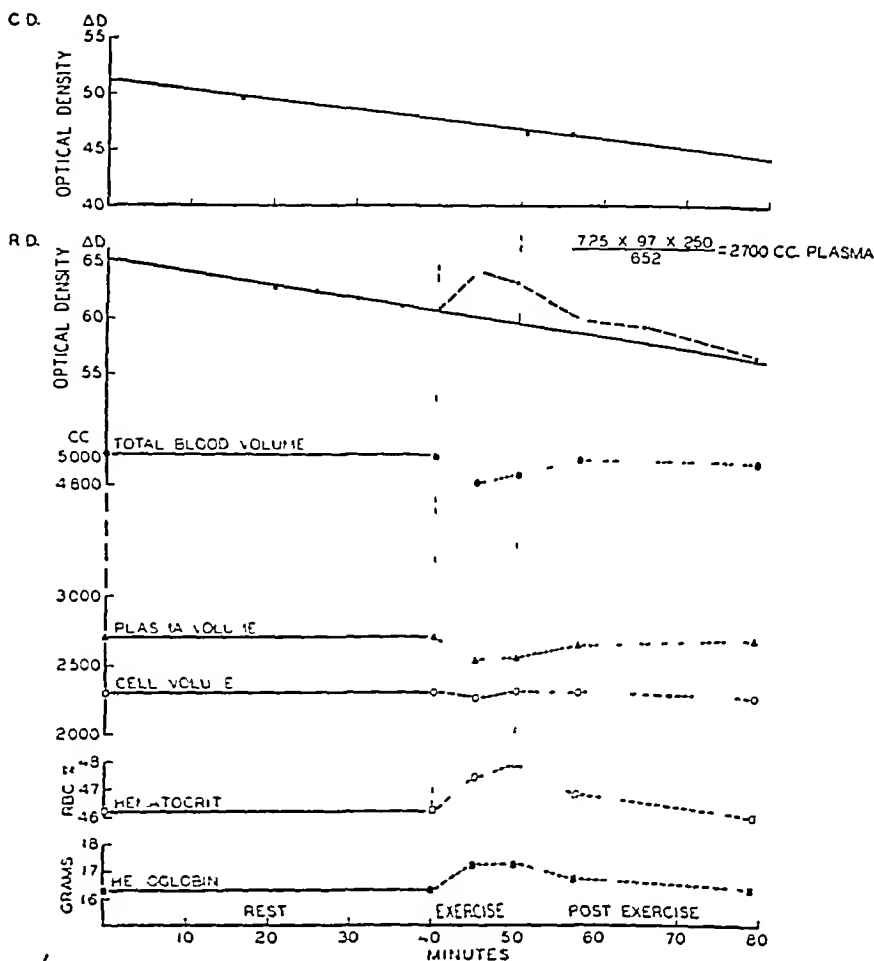


FIG. 1 THE ALTERATIONS IN THE VOLUME OF THE BLOOD DURING AND AFTER MODERATE EXERCISE IN A NORMAL INDIVIDUAL

ing the post exercise period blood was withdrawn for serum samples and relative cell volume. Circulation time and venous pressure were measured at varying intervals. The length of the experimental period was from 35 to 40 minutes.

In several experiments the type and amount of the work were changed somewhat. In one experiment (N. K.) the amount of work was doubled for 4 minutes after the usual period. In another the subject (V. D.) rode the bicycle to exhaustion. The work in two other experiments (J. C. and A. R.) consisted of going up and down steps. In still another (A. M. B.) the subject was lying in bed while the blood volume was determined after which he raised his legs above the bed and worked them vigorously for 10 minutes.

Determinations of the plasma and blood volumes were made before and following exercise on 6 normal individuals, 1 with chronic alcoholism, 1 with anorexia nervosa and 4 with compensated heart disease. In the latter group 3 of the patients had rheumatic heart disease, 1 had only involvement of the mitral valve, while the others had both aortic and mitral valves involved. The fourth patient of this group had hypertensive heart disease. All

the patients had had one or more episodes of congestive heart failure. At the time of the determinations, all of the patients were ambulatory, all complained of dyspnea on moderate exertion but only 1 had signs of congestive failure, a slight peripheral edema. The cardiac reserve of these patients was not great for all of them complained of rather severe respiratory distress on performing what is considered to be moderate exertion on the bicycle ergometer.

RESULTS

Normal subjects The characteristic changes in the plasma and blood volumes during and after moderate exercise are depicted in Figure 1 and Table I. In every case exercise was accompanied by a prompt and definite decrease in the plasma volume. The diminution in the plasma volume was associated with an elevation in arterial and venous pressures. However there was no correlation between the degree of increase in pressure and the decrease in volume. In 1 case,

TABLE I
Alterations in the volume of the blood before, during and after muscular activity (normal subjects)

	Conditions	Change in volume						Hemo- to- crit	Hemo- glo- bin	Serum pro- tein	Total circu- lating pro- tein	Vis- cosi- ty	Ven- ous pres- sure	Remarks
		Plasma		Blood		Cell								
		cc.	per cent	cc.	per cent	cc.	per cent	per cent	grams	grams per cent	grams	mm. H ₂ O		
A. M. B. January 11, 1938	Rest	3600		6460		2860		44.3	12.5			4.0	77	Sitting, bicycle ergometer; 447 kgm. per minute for 10 minutes. Normal.
	Exercise, 6 minutes	-450	-12.5	-660	-9.3	-180	-6.3	44.3	10.1			4.4		
	Exercise, 10 minutes	-430	-12.0	-620	-8.2	-190	-6.6	45.7				4.4	223	
	Post Exercise, 10 minutes	+40	+1.1	+80	+1.5	+40	+1.4	43.3	15.4			4.3	79	
A. M. B. March 29, 1938	Rest	3600		6340		2640		42.3	14.6	5.94	215	4.1		Lying in bed, working legs vigor- ously for 10 minutes, fatigued. Normal.
	Exercise, 6 minutes	-860	-24.7											
	Post Exercise, 5 minutes	-240	-6.6	-560	-10.8	-100	-3.8	48.5	15.8	6.58	210	4.5		
	Post Exercise, 8 minutes	-250	-7.0											
Post Exercise, 28 minutes	+30	+0.8	+100	+1.6	+70	+2.7	42.7	14.8	6.03	219	4.3			
R. D. January 31 1938	Rest	2700		5090		2320		46.3	16.3			4.3	77	Sitting, bicycle ergometer- 414 kgm. per minute for 10 minutes. Normal.
	Exercise, 5 minutes	-170	-6.3	-210	-4.2	-40	-1.7	47.4	17.3			4.5	114	
	Exercise, 10 minutes	-150	-5.5	-140	-2.8	+10	+0.4	47.9	17.2			4.5	84	
	Post Exercise, 7 1/2 minutes	-80	-3.0	-40	-0.8	+10	+0.4	48.5	16.3			4.3	84	
C. M. March 2, 1938	Rest	3480		6380		2900		46.4	18.5	4.72	196	4.6	33	Sitting, bicycle ergometer- 330 kgm. per minute for 10 minutes. Normal.
	Exercise, 5 minutes	-770	-22.1	-230	-4.4	-10	-0.3	47.4	18.4	6.07	198	4.7		
	Exercise, 10 minutes	-100	-2.9	-40	-0.6	+60	+2.1	46.7	18.3	6.39	216	4.7	93	
	Post Exercise, 7 1/2 minutes	-40	-1.1	+20	+0.3	+60	+2.1	46.3	18.0			4.7	93	
Post Exercise, 25 minutes	+90	+2.6	+110	+1.7	+80	+0.7	45.0	16.7	6.08	217	4.6	40		
N. K. February 22, 1938	Rest	3090		5760		2670		46.4	16.6	6.9	213	4.6		Sitting, bicycle ergometer- 428 kgm. per minute for 13 minutes, then 520 kgm. per minute for 4 minutes. Normal.
	Exercise, 5 minutes	-250	-8.1											
	Exercise, 10 minutes	-290	-9.4	-300	-5.2	-10	-0.4	48.7	17.5	7.56	212	4.8		
	Exercise, 16 minutes	-410	-12.3	-390	-6.8	+20	+0.8	50.1	17.9	7.6	204	5.3		
Post Exercise, 12 minutes	-370	-12.0												
Post Exercise, 27 minutes	-330	-10.7	-410	-7.1	-80	+3.0	48.8	18.9	7.5	207	4.7			
N. K. February 22, 1938	Rest	2930		5200		2620		47.1	18.1	6.7	220	4.8	100	Sitting, chair ergometer worked with left arm for 15 minutes. Normal.
	Exercise, 7 minutes	-120	-4.1	-270	-5.2	-40	-1.4	48.1	18.1	7.03	221	4.8	188	
	Exercise, 13 minutes	-180	-6.1	-80	-1.3	+50	+1.7	48.1	18.6			4.8		
	Exercise, 15 minutes	-200	-6.8											
Post Exercise, 13 minutes	-200	-6.8	+50	+1.5	+27	+0.7	47.5							
Post Exercise, 26 minutes	+30	+0.9	+140	+2.3	+110	+3.5	47.4		6.9	223				
V. D. June 4, 1939	Rest	3210		4800		2590		44.8	14.9	6.32	203	4.1	64	Sitting, bicycle ergometer- worked to exhaustion in 6 1/2 minutes. Normal.
	Post Exercise, 1 minute	-870	-27.1	-320	-9.2	-140	-4.8	47.9	17.2	8.07	205	4.5	118	
	Post Exercise, 8 minutes	-500	-15.6	-300	-6.2	+20	+0.7	50.7				4.3		
	Post Exercise, 11 minutes	-490	-15.3	-240	-6.1	-170	-6.7	51.0	16.3	7.34	206	5.3	30	
Post Exercise, 28 minutes	-400	-12.8	-220	-4.0	+250	+8.0	49.5	16.3						

doubling the amount of work caused a greater diminution in the plasma volume. Two individuals who did exhaustive work showed a rather marked decrease in plasma volume.

The changes in cell volume were variable. During moderate exertion the cell volume was slightly diminished in all instances at the end of 5 or 6 minutes of work. Just before work was discontinued the volume of the cells showed a very slight increase in 2 cases (R D and C M).

When the exercise was prolonged, as in the case of N K, or exhaustive work was performed, the cell volume was increased in 2 out of 3 cases. The individual (V D) performing exhaustive exercise on the ergometer showed a moderate increase in cell volume.

Due chiefly to the loss of plasma from the blood stream, the degree of diminution in the blood volume was almost parallel to that of the plasma volume. During severe exercise, however, the

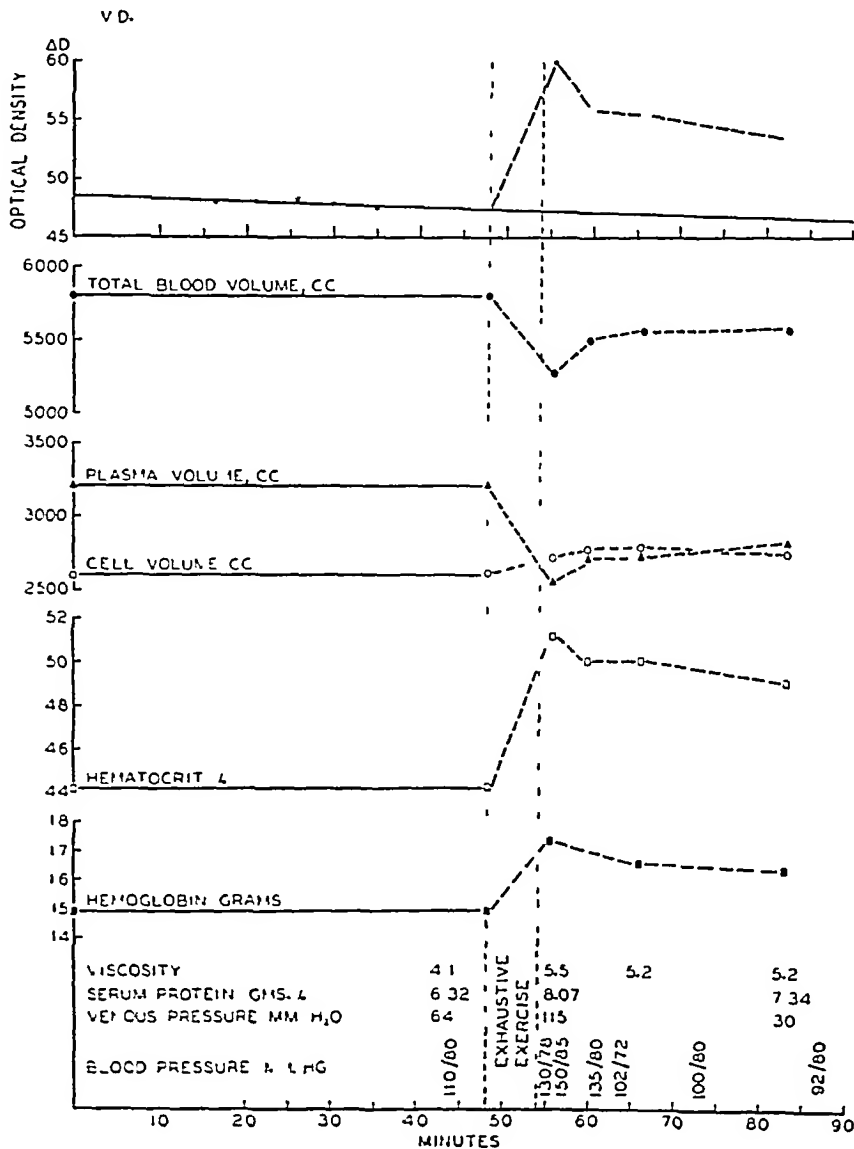


FIG. 2. THE CHANGES IN THE VOLUME OF THE BLOOD AND OTHER RELATED CIRCULATORY MEASUREMENTS DURING AND AFTER EXHAUSTIVE WORK BY A NORMAL INDIVIDUAL.

decrease in blood volume was not quite so great as that of the plasma volume because of the addition of red cells to the blood stream.

As a result of these alterations in the blood volume, the serum proteins, the hemoglobin, and the viscosity of the blood increased in proportion to the fluid loss from the blood stream. In 5 cases in which the arm-to-tongue time was measured during exercise, it was found to be decreased.

Following exercise the concentration of the dye in the serum gradually fell toward the prolongation of the disappearance slope, indicating dilution of the plasma. The plasma volume increased so that 25 minutes after exercise the volume was within +2.6 and -0.7 per cent of the initial value. Similar changes were noted in the blood volume. During the post-exercise period the alterations in the cell volume were again variable. Several cases showed a slight increase, others a slight diminution. Twenty five minutes after exertion the hemoglobin and viscosity of the blood usually reached the pre-exercise level, while the

serum protein was slightly higher than the control value.

When more severe exercise (N. K. and V. D.) was performed, the volumes of the plasma and blood were still diminished and, in 1 case (V. D.), the cell volume remained elevated 25 minutes after exercise (Figure 2). Likewise, the hemoglobin and viscosity of the blood and the serum proteins remained elevated although the venous pressure was less than that observed during the control period.

Heart disease The alterations in the blood volume due to exercise in patients with heart disease and those with poor vascular responses were very similar to those in the normal subjects (Table II). There was diminution in the plasma volume. The blood volume was not proportionally diminished because there was a slight increase in the cell volume. Here, too, the decrease in the plasma volume paralleled changes in the arterial and venous pressures. During the post-exercise period the plasma and blood volume gradually returned

TABLE II
Changes in the volume of the blood before, during and after moderate exertion in abnormal subjects

	Conditions	Change in volume						Hema- tocrit	Hemo- globin	Serum pro- teins	Total circu- lating pro- tein	Vis- co- sity	Ven- ous pres- sure	Remarks
		Plasma		Blood		Cell								
		cc.	per cent	cc.	per cent	cc.	per cent	per cent	grams	grams per cent	grams	mm. Hg		
J. C. March 30, 1930 18 years	Rest Post Exercise, 3 minutes Post Exercise, 8 minutes Post Exercise, 13 minutes Post Exercise, 25 minutes	2250 -110 -330 -290 -310	 -4.8 -10.3 -7.1 -8.5	4350 -500 -10.3 -270 -330	 -8.4 -10.3 -4.1 -7.3	2100 -90 -46 -60	 -4.3 -1.9 -2.5	32.3 41.5 40.5 40.7		6.8 8.9 7.1	231 202 209		Sitting, exercise consisted of walking up and down stairs for 7 minutes.	
M. W. March 18, 1933 44 years	Rest Exercise, 6 minutes Exercise, 10 minutes Post Exercise, 8 minutes Post Exercise, 25 minutes	2130 -110 -150 -120 -80	 -4.8 -6.2 -4.9 -2.8	4200 -300 -100 -80 -70	 -0.7 -2.3 -1.9 -1.9	1870 +80 +50 +40 -10	 +4.3 +2.7 +2.1 -0.5	42.5 45.7 45.1 44.0	14.3 15.0 15.0 14.8	6.8 7.4 7.3	168 169 173	4.1 4.3 4.1	87 95 107 89	Sitting, bicycle ergometer; 227 kpm. per minute for 10 minutes. Anorexia nervosa.
P. R. March 20, 1933 19 years	Rest Post Exercise, 3 minutes Post Exercise, 25 minutes	2620 -140 -8	 -5.3 -0.3	4600 -170	-3.3	2470 -20	-0.8	43.6 49.8 48.1	17.1 17.7 17.3	6.9 7.3	181 150	5.3 5.3 5.4		Chronic alcoholism.
O. H. February 24, 1933 48 years	Rest Exercise, 5 minutes Exercise, 10 minutes Post Exercise, 7 1/2 minutes Post Exercise, 25 minutes	2220 -280 -340 -90 -80	 -12.6 -15.3 -2.8 -3.6	4740 -20 -190 -10.8 -60	 -0.3 -3.1 -0.9	2320 +340 +160 0	 +14.8 +6.9 0	43.0 48.3 48.1 46.3	14.7 17.3 18.0 15.0	6.7 6.4 6.4 6.9	184 184 187	4.3 4.0 4.1	68 103 63 63	Sitting, bicycle ergometer; 334 kpm. per minute for 10 minutes. Rheumatic heart disease, mitral stenosis, and insufficiency
H. C. March 4, 1933 46 years	Rest Exercise, 5 minutes Exercise, 10 minutes Post Exercise, 7 1/2 minutes Post Exercise, 25 minutes	2080 -230 -280 -110 -90	 -11.1 -13.5 -2.8 -3.6	4410 -310 -100 -100 -100	 -2.9 -3.5 -1.9 -1.9	2450 +20 +10 +10 -10	 +0.8 +0.4 +0.4 -0.4	48.3 47.8 47.5 46.8	18.0 17.3 17.3 16.8	6.3 6.79 6.89	157 158 193	4.3 4.6 4.4 4.8	97 153	Sitting, bicycle ergometer; 344 kpm. per minute for 10 minutes. Rheumatic heart disease, mitral stenosis, mitral insufficiency, aortic (insufficiency aortic dilatation.
A. R. April 27, 1933 55 years	Rest Post Exercise, 1 minute Post Exercise, 14 minutes Post Exercise, 27 minutes	2430 -280 -50 +30	 -11.5 -2.1 +1.2	5210 -150 -2.4 +130	 -2.8 +1.9	2780 +130 +90	 +4.7 +3.3	44.8 44.0 45.3	16.5 16.8 16.3	7.3 7.1 6.4	218 224 221			Sitting, exercise consisted of walking up and down stairs for 8 minutes. Hypertensive heart disease. Lung clear. Right pretibial edema.

the resting values. Cases J C and W showed a more marked failure to regain necessary plasma volume level than did most the cardiac subjects. Parallel changes were in place in regard to serum proteins, hemoglobin and the viscosity of the blood. If, on the assumption that the plasma proteins not enter or leave the circulating blood during the procedures, the changes in plasma volume during the experimental period can be calculated from the values of the serum proteins, the results as obtained are in fairly good accord with those obtained directly by the dye method, except in 2 cases (C M and J C). No consistent differences between the two methods were noted which could be interpreted to indicate that protein left the blood stream during or after exercise (Table III).

TABLE III

Comparison of the values observed for the plasma protein with those calculated from the changes in total plasma volume

	Average plasma protein for experimental period		Ratio Observed Calculated	Conditions
	Observed	Calculated from change in plasma volume		
M B	67	71	0.94	Post Exercise 2 minutes
	60	59	1.02	Post Exercise 28 minutes
M	61	61	1.00	Exercise 5 minutes
	64	58	1.10	Exercise 10 minutes
	61	55	1.11	Post Exercise 25 minutes
J K	76	75	1.01	Exercise 10 minutes
	76	80	0.95	Exercise 16 minutes
	75	77	0.97	Post Exercise 27 minutes
N K.	70	70	1.00	Exercise 12 minutes
	69	67	1.03	Post Exercise 26 minutes
V D	81	80	1.01	Post Exercise 1 minute
	73	72	1.01	Post Exercise 28 minutes
J C.	69	76	0.91	Post Exercise 5 minutes
	71	75	0.95	Post Exercise 25 minutes
M W	74	73	1.01	Exercise 10 minutes
	73	70	1.04	Post Exercise 25 minutes
P B	73	73	1.00	Post Exercise 5 minutes
G H	64	64	1.00	Exercise 9 minutes
	59	58	1.02	Post Exercise 25 minutes
H C.	68	68	1.00	Exercise 10 minutes
	69	65	1.06	Post Exercise 25 minutes
A. R.	71	69	1.03	Post Exercise 1 minute
	64	62	1.03	Post Exercise 27 minutes

DISCUSSION

These results in general are at variance with those reported in the literature, except in a few in-

stances where indirect studies of the blood volume were made (4, 5). The discrepancies in the results obtained by us and those of previous investigators arise, we believe, from errors in the earlier techniques and procedures employed. The criticisms pointed out by Gregersen, Gibson, and Stead and others (19, 14) are particularly true when plasma volumes are estimated within short intervals and at times when the circulating blood volume is changing rapidly. In contrast to earlier methods, the one used in these experiments aptly lends itself to a study of changes occurring during experimental procedures. In the first place, ample time (15 to 20 minutes) is given for the dye to be completely mixed with the plasma before variations in its volume are brought about, secondly, it is not necessary to inject more dye at the time when the plasma volume is rapidly changing, and, finally, alterations produced can be followed both during and after the exercise period.

In contrast to the conflicting results reported in the literature, the observations presented above show a consistency in the direction and extent of changes of the plasma and blood volumes during and following short periods of moderate exertion in both normal subjects and individuals with heart disease. During muscular exertion the plasma volume diminishes. The decrease is proportional to the severity of the exercise. The decrease in the plasma volume is due to the sudden shift of fluid from the vascular system to the interstitial fluid and the active muscles. This transfer of fluid is attributed to the rise in systemic pressure and the hyperemia of the muscle which results in a rise of the local and capillary pressure. This in turn leads to a disturbance in the filtration-absorption equilibrium of fluids in the capillaries (20). As the blood passes through the active muscles, more fluid is forced out into the tissues than can be immediately absorbed. Some of the fluid presumably goes into the muscle cells (4, 20, 21). After cessation of exercise, the process reverses.

Consistent findings were also observed in the cell volume as a result of exercise. No new cells were added to the circulation during moderate exertion in normal individuals. Therefore, it is evident that the increase in the red blood cells and hemoglobin during work is due to a concentration of the blood. Furthermore, these observations support the view that the increased volume of the

supplied to the active muscles is brought mainly by a redistribution rather than by an increase in the volume of the circulating blood. When, however, the amount of work is greater, the strain on the capacity of the subject—whether normal or with diminished cardiac reserve—then not only is the plasma volume diminished but also cell volume is increased. But even under these circumstances the diminution in the plasma volume is always more marked than the increase in cell volume (Case V D).

There is some evidence to indicate that the moderate increase in cell volume during exhaustive exercise may be due to the addition of red cells from the blood reservoirs to the circulation. In animals there is ample proof that exercise causes the spleen to contract and discharge cells into the blood stream (3). It has been shown that this contraction is mediated through the nervous system and that either lack of oxygen or an increased secretion of adrenin may act as the stimulus. However, in man, the depot function of the spleen and other organs may be relatively unimportant, since during exercise when presumably the reserves are called out, the circulating cell volume is not greatly increased even when the exercise is severe. From the experimental data available it appears that the stimulus for the contraction of the blood depots during severe exercise in man is due to the sympathico-adrenal mechanism rather than lack of oxygen (22). It has been shown that the subcutaneous administration of epinephrine is followed by a prompt rise in the cell volume (23) while lack of oxygen requires a relatively long time (several hours to days) for it to become effective in increasing the cell volume (24). Exhaustive locomotor activity by normal subjects and moderate activity by individuals with poor vascular control are probably associated with emotional excitement, an excellent stimulus for increased secretion of adrenin (22).

Finally, it must be pointed out that the cell volume is not measured directly by this method. The calculations of the blood and cell volumes are based on the ratio of cells to plasma as drawn from the antecubital vein. There is sufficient evidence to indicate that the red cells and the plasma are not thoroughly and uniformly mixed within the vascular system (25). Until more data are available concerning the alteration that may take

place in the ratio of cells to plasma in various parts of the vascular system during exercise, one does not know how much reliance to place upon the above changes in cell volume.

SUMMARY AND CONCLUSIONS

Determinations of the volume of the blood were made at rest and variations in this volume were followed during and after varying grades of exercise in normal subjects and in individuals suffering with cardiac disease. Additional observations included measurements of the blood hemoglobin and viscosity, serum proteins, and venous pressure. The results of this investigation lead to the following conclusions:

- 1 In normal individuals during moderate exertion there is a prompt and definite decrease in the plasma volume, accompanied by a corresponding decrease in the blood volume while the changes in the cell volume are variable though slight. These changes are associated with an increase in the blood hemoglobin and viscosity, the serum proteins and the venous and arterial pressures. Following exercise the plasma volume gradually increases and 25 minutes after exercise the plasma volume, blood hemoglobin and serum proteins reach the pre-exercise values.

- 2 During exhaustive exercise in normal subjects there is a further decrease in plasma volume accompanied by a moderate increase in the cell volume. Twenty five minutes after the cessation of exercise the plasma volume is still diminished and the blood hemoglobin and serum proteins are increased.

- 3 In patients with compensated heart disease the changes in the blood volume during and following exercise are similar to those of normal subjects.

- 4 The increase in red blood cells and hemoglobin concentration resulting from exercise is brought about mainly by passage of protein poor fluid from the vascular system into the interstitial spaces. It is only during severe or exhaustive exercise that new cells are added to the circulating blood.

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If, on the assumption that the plasma proteins do not enter or leave the circulating blood during these procedures, the changes in plasma volume during the experimental period can be calculated from the values of the serum proteins, the results thus obtained are in fairly good accord with those obtained directly by the dye method, except in 2 cases (C M and J C). No consistent differences between the two methods were noted which could be interpreted to indicate that protein left and entered the blood stream during or after exercise (Table III).

TABLE III

Comparison of the values observed for the plasma protein with those calculated from the changes in total plasma volume

	Average plasma protein for experimental period		Ratio Observed Calculated	Conditions	
	Observed	Calculated from change in plasma volume			
A M B	6.7	7.1	0.94	Post Exercise	2 minutes
	6.0	5.9	1.02	Post Exercise	28 minutes
C M	6.1	6.1	1.00	Exercise	5 minutes
	6.4	5.8	1.10	Exercise	10 minutes
	6.1	5.5	1.11	Post Exercise	25 minutes
N K	7.6	7.5	1.01	Exercise	10 minutes
	7.6	8.0	0.95	Exercise	16 minutes
	7.5	7.7	0.97	Post Exercise	27 minutes
N K.	7.0	7.0	1.00	Exercise	12 minutes
	6.9	6.7	1.03	Post Exercise	26 minutes
V D	8.1	8.0	1.01	Post Exercise	1 minute
	7.3	7.2	1.01	Post Exercise	28 minutes
J C.	6.9	7.6	0.91	Post Exercise	5 minutes
	7.1	7.5	0.95	Post Exercise	25 minutes
M W	7.4	7.3	1.01	Exercise	10 minutes
	7.3	7.0	1.04	Post Exercise	25 minutes
P B	7.3	7.3	1.00	Post Exercise	5 minutes
G H	6.4	6.4	1.00	Exercise	9 minutes
	5.9	5.8	1.02	Post Exercise	25 minutes
H C.	6.8	6.8	1.00	Exercise	10 minutes
	6.9	6.5	1.06	Post Exercise	25 minutes
A R.	7.1	6.9	1.03	Post Exercise	1 minute
	6.4	6.2	1.03	Post Exercise	27 minutes

DISCUSSION

These results in general are at variance with those reported in the literature, except in a few in-

stances where indirect studies of the blood volume were made (4, 5). The discrepancies in the results obtained by us and those of previous investigators arise, we believe, from errors in the earlier techniques and procedures employed. The criticisms pointed out by Gregersen, Gibson, and Stead and others (19, 14) are particularly true when plasma volumes are estimated within short intervals and at times when the circulating blood volume is changing rapidly. In contrast to earlier methods, the one used in these experiments aptly lends itself to a study of changes occurring during experimental procedures. In the first place, ample time (15 to 20 minutes) is given for the dye to be completely mixed with the plasma before variations in its volume are brought about; secondly, it is not necessary to inject more dye at the time when the plasma volume is rapidly changing; and, finally, alterations produced can be followed both during and after the exercise period.

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- 3 In patients with compensated heart disease the changes in the blood volume during and following exercise are similar to those of normal subjects.

- 4 The increase in red blood cells and hemoglobin concentration resulting from exercise is brought about mainly by passage of protein poor fluid from the vascular system into the interstitial spaces. It is only during severe or exhaustive exercise that new cells are added to the circulating blood.

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A STUDY OF THE EFFECTS OF SULFANILAMIDE ON ACID-BASE METABOLISM^{1, 2}

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That there is a reduction of the CO_2 content of the serum of patients receiving sulfanilamide was pointed out by Southworth (1). This finding has been regularly encountered by other observers. Usually hyperpnea of varying degrees is present. The development of severe acidosis is, however, very rare. That the urine becomes strongly alkaline at the same time was pointed out by Basman and Perley (2). Strauss and Southworth (3) described a large amount of fixed base in the form of bicarbonate in the urine. Marshall, Cutting and Emerson (4) demonstrated that large doses of the drug (1 or 2 grams per kgm.) cause very severe acidosis in dogs. These findings produced the inference that the change in breathing was compensatory for bicarbonate reduction in the plasma. Recently, however, Hartmann, Perley and Barnett (5) have advanced the hypothesis that the clinical and chemical findings can best be explained on the basis of an alkalosis caused by a primary hyperventilation. Their evidence was the demonstration in four subjects of a slight rise in serum pH in the presence of considerable reduction of serum bicarbonate and secretion of a strongly alkaline urine. According to this view, the removal of fixed base in urine, with consequent reduction of plasma bicarbonate is a secondary and compensatory event. That such an adjustment may take place in response to reduction of plasma carbonic acid by hyperventilation is shown by the results of voluntary overbreathing experiments (6), although it should be noted that in these experiments the removal of bicarbonate in urine occurs in the presence of a much more extensive increase in serum pH than Hartmann found following sulfanilamide administration.

This paper presents the results of a further examination of the changes in acid base excretion and in the electrolyte structure of the blood serum in the presence of sulfanilamide administration and of the relationship of increased lung ventilation to these changes. These two items of inquiry were approached by different paths. The results obtained will therefore be described separately.

ACID-BASE CHANGES IN URINE AND IN BLOOD SERUM

The information sought was a description of changes in acid base excretion in urine during periods of sulfanilamide administration sufficiently quantitative to permit correlation with measurements of alterations in the usual electrolyte structure of the serum.

Plan of study

In order to provide a stationary intake of the individual electrolytes the patients were maintained on a rigidly constant metabolic regime. The diet in each case was determined by usual food habits and then identical weighed portions of each article of food were eaten daily. A constant fluid intake was maintained at a liberal level. After a week on the constant diet, consecutive 24-hour collections of urine were begun and continued throughout the period of study which was divided into a foreperiod of 6 to 8 days, a period during which the patient received sulfanilamide every 4 hours, followed by an afterperiod of about one week.

Two patients were submitted to this plan of study. Subject W. P. was a 26-year-old gardener well in all respects except for gonorrheal arthritis of the right knee of 4 months duration. The joint became entirely well during treatment. The period of sulfanilamide administration during which he received 1.33 grams of the drug every 4 hours extended over 28 days. Subject E. C. was given 60 grams of sulfanilamide over a period of 10 days.

Analytical methods

The 24-hour collections of urine were preserved with toluol. A small portion of each voiding was collected under oil for measurement of pH which was done

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² The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

colorimetrically by the method of Hastings, Sendroy and Robson (7) Immediately on completion of each 24-hour urine collection, the measurement of ammonia was carried out by the method of Folin as modified by Van Slyke and Cullen (8) The other analytical methods used were as follows sodium, Butler and Tuthill (9), potassium, Fiske and Litarczek (10), total base, Fiske (11), calcium, Fiske and Logan (12), chloride, Eisenman (13), phosphorus, Fiske and Subbarow (14), sulfanilamide, Marshall (15), total nitrogen, the Kjeldahl method The blood was collected under oil and the serum separated anaerobically as soon as clotting permitted. The CO_2 content of the serum was determined by the method of Van Slyke and Neill (16) Serum pH was measured colorimetrically by the method of Hastings and Sendroy (17) In obtaining measurements of other components of serum, the methods already cited were used.

Results

The measurements obtained from W P over the 42 days of study are displayed graphically in Figure 1 Several features of the data which describe the excretion of Na, Cl, K and NH_4 in the urine are readily apparent The most prominent one is a very large rise in the excretion of sodium during the first day of administration of sulfanilamide Over the remainder of the prolonged period of study the daily excretion of Na, although quite widely fluctuant, approximates the roughly defined foreperiod level Immediately following the cessation of sulfanilamide administration, a progressive and extensive reduction of Na

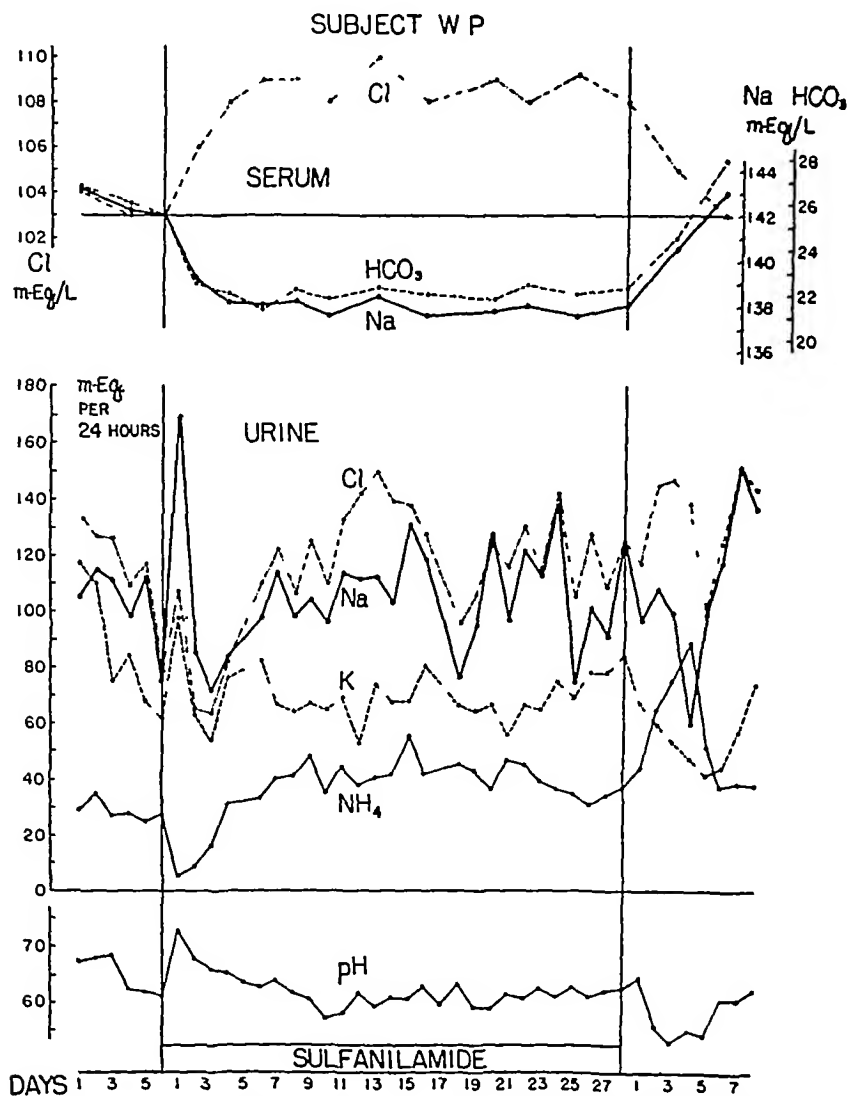


FIG 1 DATA OBTAINED DURING A 28-DAY PERIOD OF SULFANILAMIDE ADMINISTRATION

excretion occurs over a 4-day period, which is then followed by a stepwise return to the region of the foreperiod level. The initial rise in Na is accompanied to only a slight extent by Cl, nor does Cl excretion in the afterperiod follow the large reduction in the excretion of Na. Thus, during the first day of sulfanilamide administration there is removal of Na in large excess over Cl and in the afterperiod the reverse event, a much more extensive removal of Cl than of Na. The measurements of K describe changes in excretion level in the same directions as Na but of considerably less extent. The NH_4 values show at the outset

of the sulfanilamide period a brief but extensive recession and in the afterperiod a large rise, in other words an inverse relationship to the two large components of fixed base excretion, Na and K. As would be expected, urine pH displays changes reciprocal with respect to NH_4 .

The measurements of concentration of Na, HCO_3 , and Cl in the blood serum are plotted in the upper section of the chart with reference to the values found on the day preceding the period of sulfanilamide administration and they clearly describe two events. At the outset of the sulfanilamide period the (Na) falls rapidly and at

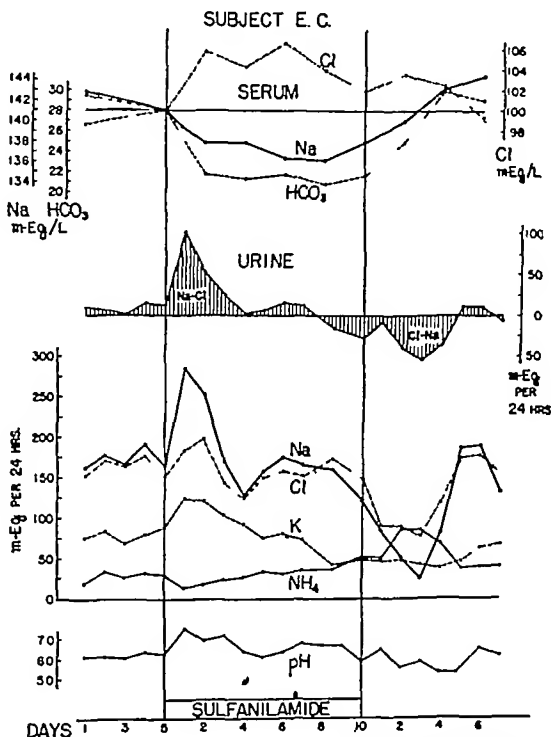


FIG. 2. DATA OBTAINED DURING A 10-DAY PERIOD OF SULFANILAMIDE ADMINISTRATION

In the middle curve the shaded area above the base line indicates the difference of sodium excretion-chloride excretion while that below indicates chloride excretion-sodium excretion, demonstrating the lack of companionship between these two ions.

the end of the third day is about 4 m eq per liter below the initial value. This level is then approximately sustained until sulfanilamide is removed, whereupon (Na) returns in the course of 6 days to a usual value. These changes in (Na) are accompanied by closely equivalent changes in (HCO_3). The other event is a somewhat larger change of (Cl) in the other direction, producing an increase of about 6 m eq. Here again there is the feature of rapid approach to the new level which is then held fairly steadily over the sulfanilamide period.

The data from the other subject, E C, are recorded in Figure 2 and also in Table I. As may be seen in Figure 2, the features of change in the daily excretion values for Na, Cl, K, and NH_4 and in the concentrations of Na, HCO_3 and Cl found in the serum of W P are again described. The relationship of Na and Cl excretion is, however, much more clearly defined by the measurements

obtained from this subject. To make quite clear the extent of disruption of the companionship of Na and Cl, the values for the initial excess of removal of Na over Cl and the subsequent larger excretion of Cl than of Na are plotted on the chart.

Discussion

If it be assumed that the extrarenal removal of Na, K and Cl by way of the skin and in the feces is a small fraction of the total excretion and has a fairly constant value, fluctuations in the daily quantity in urine, in the presence of a stationary intake, may be taken as measuring gain or loss from the body.

This premise is used with the reservation that the measurements of gain or loss of Na or Cl which it provides must be regarded as rather rough approximations. The daily excretion values for these extracellular electrolytes have

TABLE I
E C—Urine data

Date	Volume	Total base	Sodium	Potassium	Calcium	Average pH	Phosphorus	Chloride	Ammonia	Sulfanilamide	Total N
		<i>total m Eq</i>	<i>total m Eq</i>	<i>total m Eq</i>	<i>total m Eq</i>		<i>total m Eq *</i>	<i>total m Eq</i>	<i>total m Eq</i>	<i>total grams</i>	<i>grams</i>
March 18	2540	258.5	162.1	75.3	11.1	6.15	40.7	151.9	23.57		12.193
March 19	2795	270.9	178.4	82.59	10.5	6.18	29.71	171.2	34.0		11.698
March 20	2198	261.7	166.2	69.3	9.9	6.10	32.1	165.9	25.9		10.427
March 21	2385	274.0	191.5	84.5	9.5	6.40	40.1	176.0	30.64		11.350
March 22	2105	254.2	164.6	87.6	8.9	6.30	43.2	151.7	29.09		11.311
SULFANILAMIDE 6.0 GRAMS DAILY BEGUN											
March 23	3000	415.6	284.3	123.53	11.1	7.50	63.7	183.7	12.84	1.436	12.387
March 24	2965	380.8	251.6	121.0	11.4	6.95	50.9	198.5	17.35	3.882	12.420
March 25	2780	277.9	169.2	103.6	12.8	7.17	54.4	143.14	22.6	4.099	12.638
March 26	2330	224.1	127.8	91.71	12.8	6.40	34.3	124.1	26.9	4.093	11.943
March 27	2540	249.8	157.6	76.1	12.7	6.17	30.6	150.2	34.2	4.461	13.270
March 28	2540	263.7	176.3	81.6	14.5	6.40	33.2	159.3	31.1	4.258	12.031
March 29	2180	244.1	165.7	72.8	14.6	6.82	45.2	152.6	36.3	4.349	13.024
March 30	2430	298.9	216.76	62.4	16.6	6.73	34.3	211.9	46.14	5.167	12.954
March 31	1830	158.7	102.9	22.4	11.8	6.72	29.2	135.7	28.6	4.392	11.722
April 1	2720	221.5	122.2	48.9	13.4	5.92	25.6	148.8	52.06	5.195	12.385
SULFANILAMIDE STOPPED											
April 2	1985	158.8	113.0	48.0	10.8	6.55	37.7	91.08	47.64	3.819	12.491
April 3	1545	106.6	50.91	51.8	10.4	5.60	37.8	90.64	85.7	1.328	14.080
April 4	1545	83.7	24.3	43.2	7.9	5.92	35.1	78.25	84.5	0.315	12.690
April 5	1550	140.6	81.7	39.5	8.5	5.32	40.0	118.0	68.8	0.084	10.856
April 6	2075	246.7	187.4	46.6	8.3	5.32	29.8	175.2	38.4	trace	9.844
April 7	2460	255.1	189.0	63.8	9.4	6.55	26.3	177.3	38.9	0	9.732
April 8	2420	255.3	145.6	65.8	8.3	6.22	45.5	152.6	39.94	0	8.906

* These values were calculated from the total phosphorus in mols from the pH by the use of the equation

$$\text{pH} = 6.66 + \log \frac{\text{HPO}_4^-}{\text{Total P} - \text{HPO}_4^-}$$

been found to oscillate considerably even when intake is accurately constant (18). However, in the presence of wide change in excretion, estimation of gain or loss obtained from an approximately defined foreperiod level may be accepted as dependably descriptive. On this basis the most conspicuous finding from the urine data is a large loss of Na at the outset of the sulfanilamide period which is to only a slight extent accompanied by an increase in chloride excretion. Along with this event the serum data describe a reduction of (Na) and an increase of (Cl). Rough calculation of the relationship of Na loss to (Na) reduction in the serum produces the information that the loss is much larger than the fall in concentration in the serum describes. Using the data from E. C. (Table I), and taking the average of the measurements of Na over the foreperiod as representing the level of excretion in urine which sustains Na balance in the body, a loss of 190 m. eq. is found over the first 2 days of sulfanilamide administration. Reduction of (Na) in the serum was 33 m. eq. per liter. Total extracellular fluid, on assumption that it amounts to about 20 per cent of body weight, was 12 liters for this subject. Thus a loss of 40 m. eq. of Na would account for the fall in plasma (Na). On the premise that Na is held in the body only in extracellular fluid, the remainder of the Na loss in urine, 150 m. eq., must be accompanied by an equivalent withdrawal of extracellular water. Since the water loss is presumably at the expense of interstitial fluid water in which the normal value for (Na) may be taken as 147 m. eq. per liter, the estimated reduction of extracellular fluid volume in this subject amounts to about one liter. Incidentally this provides explanation of the rise of (Cl) found in the blood serum. The expected rise may be roughly estimated. The value for (Cl) in the serum at the beginning of the sulfanilamide period was 100 m. eq. per liter so that the 12 liters of extracellular fluid contained 1200 m. eq. of Cl. Excretion of Cl in the urine, above the foreperiod level, was 55 m. eq. over the first 2 days. The increased (Cl) caused by reduction of extracellular fluid volume to 11 liters should therefore be $1200 - 55/11 = 104$ m. eq. per liter, which is in the neighborhood of the value found, 105.8 m. eq. per liter. This objective explanation

of the process of increase in plasma (Cl) does not, of course, tell us why renal control permits it.

Another clearly evident finding is an increased removal of potassium. Estimated from the foreperiod level of excretion, this loss of K for the first 2 days of the sulfanilamide period is 85 m. eq. The measurements of (K) in the serum show no appreciable change. Loss of one liter of extracellular fluid would remove only about 4 m. eq. of K. The increased excretion must therefore be derived from intracellular fluid where K is the largest component of the total base value. The observed reduction of (Na) in extracellular fluid of 33 m. eq. per liter may reasonably be supposed to make necessary a corresponding reduction of (K) in the adjacent fluid. If the intracellular fluid volume for this subject be taken as 30 liters (50 per cent of body weight), then the expected removal of K would be $30 \times 33 = 99$ m. eq. The loss of K therefore probably does not involve a reduction of intracellular fluid volume.

From the plasma data recorded in Figures 1 and 2, it may be seen that the changes in (HCO_3) and (Cl) are roughly reciprocal. Taken by themselves they would suggest very strongly that the bicarbonate reduction caused by the administration of sulfanilamide could be described as a "chloride acidosis." The close correspondence of the reductions of (Na) and of (HCO_3) makes it clear, however, that bicarbonate is lowered by removal of BHCO_3 and not by a process of displacement of the anion HCO_3 by Cl. The increase in (Cl) is therefore entirely gratuitous as an explanation of bicarbonate reduction and is also mysterious as regards its accommodation in the acid base framework of the serum. As shown by the total base measurements in Table I, and also those of K and Ca, there is no replacement of the Na reduction by extension of the other components of serum base. As regards sulfanilamide itself, if it be assumed that this substance behaves as base and crediting it with divalency the concentration measured in the serum would not cover more than a small fraction of the (Cl) increase. Among the anion components protein is the only one large enough to provide place by recession for the approximately 5 m. eq. per liter increase of (Cl). Since there is no reduction of the concentration of protein, base could be supplied only by alteration of its usual base equiv- in

some way related to the presence of sulfanilamide. That such an event occurs is the only surmise that the authors are able to devise from the data at hand. The manner of the covering of the (Cl) increment therefore awaits further investigation.

The changes in acid-base excretion in urine and the accompanying changes in the electrolyte structure of the plasma which these data describe suggest at first glance a disability of renal control in the presence of sulfanilamide. This surmise, however, is not altogether suited by the outstanding feature of these findings, namely, an initial large removal of sodium which is then followed by a steadily sustained excretion at the foreperiod level. The alternative explanation that the changes in the electrolyte structure of the serum represent adjustments which are made necessary by the presence of sulfanilamide and which are thereafter accurately sustained by the kidney at least deserves consideration, the authors believe, in spite of the implied offense to the threshold conception of renal control. The curious inverse change in the serum levels for (Na) and (Cl) is not to their minds readily referable to an error in tubular function. The authors, however, admit that these reflections scarcely constitute an argument. It is perhaps worth noting that these effects from sulfanilamide administration bear a resemblance in some respects to those found by Gamble, Blackfan and Hamilton (19) for the so-called acid-producing salts. There is the same removal of Na and K with an accompanying loss of body water and the same large increase in serum (Cl). They record the curious finding that this increase in (Cl) is apparently not a direct consequence of the ingestion of calcium chloride or of ammonium chloride since it also occurs to the same extent following the administration of ammonium sulfate. There is no appreciable change in plasma fixed base so that, in the case of these salts, the reduction of plasma HCO_3 , which occurs is clearly referable to the increase in (Cl).

THE RELATIONSHIP OF HYPERPNEA AND BICARBONATE REMOVAL IN URINE FOLLOWING INGESTION OF SULFANILAMIDE

The finding, following sulfanilamide administration, of a removal of Na in excess over Cl in

the urine, reduction of serum bicarbonate and increased pulmonary ventilation produces the entertaining question of the causal relationship of these events. Does the sequence of these changes begin with the alteration of breathing or with the removal of fixed base? The usual rôle of hyperpnea as an adjustment of carbonic acid in the plasma in the presence of bicarbonate reduction points to the base loss in the urine as the initial event. Overbreathing experiments, however, have shown that primary reduction of carbonic acid in the plasma is followed by a removal of bicarbonate in the urine, and Hartmann's hypothesis is that the first step in sulfanilamide effect is hyperpnea produced presumably by action of the drug on the respiratory center. The essential item of Hartmann's evidence consists in demonstration of an increase in plasma pH, indicating a primary alkalosis for which bicarbonate reduction by removal in urine may be regarded as compensatory. The change in pH which Hartmann reports is not, however, in most instances of a convincing magnitude. Here it may be pointed out that since pH is in process of adjustment, whether by removal of bicarbonate in urine or by increased ventilation of the lungs, large departures from its normal value would not be expected. It would therefore seem desirable to measure other factors in the situation which should exhibit a greater width of change than the closely guarded pH value. Moreover, the question of the sequential relationship of the alteration in breathing, the reduction of plasma bicarbonate, and the removal of fixed base in the urine is obviously answerable if the position in time of these events following the administration of sulfanilamide can be clearly defined. To this end observations were obtained from two subjects.

Plan of experiments

The subjects were healthy young male adults. The respiratory data obtained were the rate of lung ventilation, O_2 consumption, CO_2 production and alveolar CO_2 tension³. In arterial serum, pH and CO_2 content were measured, and CO_2 tension was calculated. In the urine, pH and the rate of removal of bicarbonate were determined. These

³ We are indebted to Dr John H Talbott for these determinations.

data were obtained directly before and at several 1 to 2 hour intervals after administration of a single large dose of sulfanilamide. The spirometer collections were over 50-minute periods just preceding the taking of serum samples. In addition to the two sulfanilamide experiments, a third set of observations was obtained from one of the subjects at the beginning and at the end of a 50-minute period of voluntary overbreathing.

Results and discussion

The data from these experiments are recorded in Table III and are also shown graphically in Figure 3. The results of the two sulfanilamide experiments are in good agreement. The rise in serum pH described by Hartmann was not found in these two subjects. Actually in this early stage of sulfanilamide effect a fall of pH was observed, but of such slight extent that its significance is doubtful. The measurements of urine bicarbonate show a nearly constant rate of removal which, moreover, has about the same value for both subjects. Since the rate found from

urine collected over the first period following sulfanilamide administration (recorded on the chart

TABLE II
E. C.—Serum data

Date	Total base	Sodium	Potassium	Calcium	Phosphorus*	Chloride	Carbon dioxide	Sulfanilamide
	m.eq per liter	m.eq per liter	m.eq per liter	m.eq per liter	m.eq per liter	m.eq per liter	total m.l.	mgm per 100 cc
March 18	152.2	142.8	3.795	4.6	2.32	101.8	29.3	0
March 22	150.7	141.2	4.113	4.87	2.39	99.8	28.2	0

SULFANILAMIDE BEGUN

March 23		139.5						3.45
March 24	148.8	137.9	4.18	4.68	2.37	105.8	21.8	4.48
March 25	148.5	138.0	4.41	4.56	2.22	104.3	21.4	6.29
March 26	144.5	136.3	4.16	4.63	2.06	106.6	21.7	5.26
March 30	145.5	136.0	3.88	4.68	1.97	103.9	20.8	6.31
April 1	147.8	138.0	4.12	4.68	2.15	102.0	21.6	5.90

APRIL 2—SULFANILAMIDE STOPPED

April 3	151.8	140.1	4.05	4.34	1.97	103.64	24.9	±
April 5	155.0	143.2	4.40	4.57	1.90	102.36	29.9	0
April 7	153.8	144.4	4.76	4.60	1.86	99.4	29.0	0

* Calculated for pH 7.4 by the equation

$$7.4 = 6.6 + \log \frac{\text{HPO}_4^-}{\text{Total P} - \text{HPO}_4^-}$$

TABLE III

Data obtained in experiments relating hyperpnea and bicarbonate removal in the urine

SULFANILAMIDE EXPERIMENT I E. J.

Time	Respiration					Serum (arterial)			Urine			
	Ventilation	CO ₂	O ₂	R.Q.	Alveolar CO ₂	pH	pCO ₂	HCO ₃	Volume	pH	HCO ₃	HCO ₃
	liters per minute	cc. per minute	cc. per minute		mm. Hg		mm. Hg	m.eq per liter	cc.		total m.eq	m.eq per minute
7.35 a.m.	7.80	245	280	0.87	40.6	7.41	41	26.9	50	6.6	1.58	0.032
10.25 a.m.	6.84	220	273	0.81	39.3	7.38	39	23.8	200	7.8	33.41	0.197
12.30 p.m.	7.80	229	293	0.78	35.6	7.38	35	21.3	420	8.0	24.20	0.194
3.45 p.m.	9.26	238	318	0.74	36.4	7.40	35	22.4	120	8.1	26.50	0.135

13.3 grams sulfanilamide and 100 cc. H₂O at 9.55 a.m.

SULFANILAMIDE EXPERIMENT II R. M.

7.23 a.m.	4.64	162	277	0.75	41.7	7.42	40.3	26.4	51	5.5	0.10	0.002
8.50 a.m.	4.66	164	218	0.75	36.7	7.38	40.2	25.8	410	7.3	17.22	0.198
10.12 a.m.	4.98	166	221	0.75	35.2				350	7.5	15.60	0.190
11.25 a.m.	5.25	168	227	0.74	35.4				210	7.7	13.89	0.166
12.30 p.m.	5.42	165	227	0.72	35.2	7.38	35.3	22.2	95	7.8	10.77	0.026

13.3 grams sulfanilamide and 200 cc. H₂O at 8.35 a.m.

OVERBREATHING EXPERIMENT R. M.

7.35 a.m.	5.70	167	215	0.78	33.1	7.40	40.0	21.2	165	5.5	0.26	0.005
8.30 a.m.	8.54	206	206	1.00	20.7	7.57	22.0	19.5	210	6.9	3.92	0.071

50-minute voluntary hyperventilation begun at 7.37 a.m.

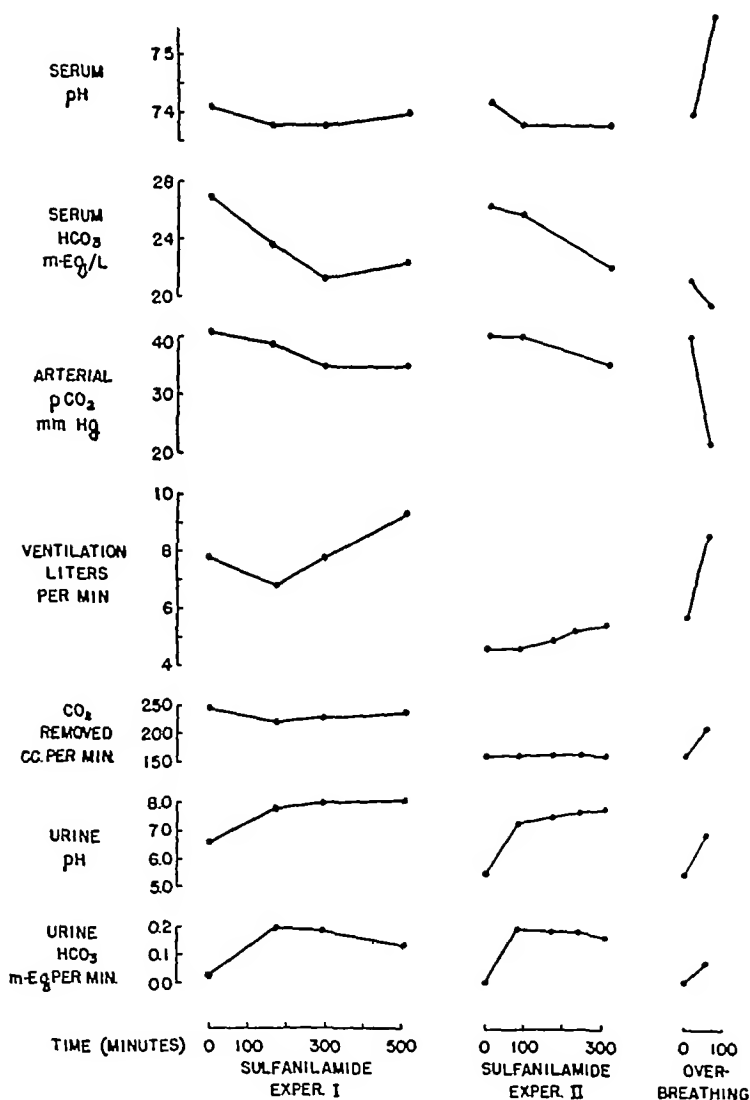


FIG 3 DATA IN TABLE III DEMONSTRATING THE DIFFERENCE BETWEEN THE SULFANILAMIDE EXPERIMENTS AND THE OVERBREATHING EXPERIMENT

at the end of the period) has approximately the same value as in the subsequent periods, it is evident that establishment of this rate of removal is an almost immediate effect of sulfanilamide. The change in urine pH corresponds. Serum bicarbonate is found to decline over a period of 5 hours when, according to the longer experiment (I), it becomes roughly stationary. The measurements of the volume of lung ventilation have a critical significance. As may be seen in Figure 3, no increase in ventilation is found at the end of the first period following sulfanilamide. Gradual development of hyperpnea in these two subjects is de-

scribed by the subsequent measurements. Since the urine data demonstrate that the process of bicarbonate removal is fully established during the first period following sulfanilamide, priority of this event would seem to be clearly indicated. This inference is supported by the measurements of arterial CO₂ tension. By the end of the first period pCO₂ in Experiment I has fallen only slightly and in Experiment II is found unchanged. A discordant item in the evidence must be admitted. The measurements of alveolar CO₂ tension show a definite reduction at the end of the first period in the second experiment and are

therefore in direct disagreement with the measurements of ventilation and of arterial $p\text{CO}_2$. The authors are unable to account for this discrepancy. A finding of incidental interest is that the rate of CO_2 removal by the lungs is not measurably increased by the gradual development of hyperpnea. Appreciable reduction referable to CO_2 removed in the urine as bicarbonate would not be expected since this quantity is relatively minute, amounting to about 6 cc. per minute.

The defects of resemblance of the data found following sulfanilamide administration to those data produced by the 50-minute overbreathing experiment, which describe a situation of primary alkalosis, are clearly evident in Figure 3. The measurements of serum pH and of arterial $p\text{CO}_2$ show that the expected change of these values in the presence of increased ventilation of the lungs is rapid and extensive and the urine data demonstrate that adjustment by removal of bicarbonate is under way by the end of the 50-minute period. Here, however, it may be noted that in the presence of great change in ventilation the rate of removal of bicarbonate is much smaller than is found in the sulfanilamide experiments in the absence of appreciable change in ventilation. This fact suits the secondary position of bicarbonate removal in the overbreathing experiment and points clearly to its primary significance following sulfanilamide.

The total evidence of the data from these experiments thus quite definitely indicates that the hyperpnea which develops following sulfanilamide administration should be regarded as an adjustment to an initial removal of fixed base in the urine.

SUMMARY

Over a period of continued administration of sulfanilamide, several alterations of acid base metabolism are found to occur. There is a large loss of sodium accompanied by a smaller loss of potassium and little or no loss of chloride ion. The concentration of sodium in blood plasma is reduced to the extent of 4 to 5 m eq per liter. There is no replacement of this deficit by extension of the other components of total base. The loss of sodium is much larger than the reduction of its serum concentration indicates and therefore presumably involves a considerable withdrawal of

extracellular water. The quantity of potassium lost suits the hypothesis of a reduction of total base concentration in intracellular fluid to the extent caused in extracellular fluid by the recession of sodium. The fall of sodium concentration in the plasma corresponds closely with and explains a reduction of bicarbonate. The concentration of chloride ion, on the other hand, is increased to the extent of 5 to 6 m eq per liter above the initial value. This elevation agrees in extent with a reduction of extracellular fluid volume estimated from the sodium loss. The accommodation, in terms of base equivalence, of this extension of chloride ion in the presence of an actual reduction of total base is unexplained. An outstanding feature of these alterations of acid base excretion and of the electrolyte structure of the plasma is that they occur rapidly (within 1 to 3 days) at the outset of the period of sulfanilamide administration. Electrolyte exchange and the altered values in the plasma are then steadily sustained until sulfanilamide is removed. The initial losses of sodium and potassium are then rapidly recovered and the normal electrolyte pattern of the plasma is restored.

Observations of respiratory change, serum alterations and the removal of sodium in the urine at short (1 to 2 hour) intervals following ingestion of a single large dose of sulfanilamide clearly describe the entrance of bicarbonate into the urine as an almost immediate event which is followed by a gradual development of increase in lung ventilation. The hyperpnea observed during sulfanilamide therapy may therefore be regarded as a secondary event compensatory to plasma bicarbonate reduction caused by removal of fixed base in the urine.

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SERUM URATE IN RELATIVES OF GOUTY PATIENTS¹

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Though the hereditary nature of gouty arthritis has been recognized for centuries, systematic studies of afflicted families are rare. Of several items which are pertinent to such studies, there are at least two significant ones. These concern (a) the hereditary transmission of gout and (b) the pathogenesis of its clinical manifestations. If the hereditary data are obtained from routine clinical records without painstaking study of the living relatives, the familial incidence appears to be low. Diligent medical and social investigation of gouty families, however, discloses evidence which indicates a high familial incidence and supports the hypothesis that gout is an hereditary malady (1, 2, 3, 4, 5). Such a survey which has been conducted by us has shown in addition that an elevated serum urate may be observed in relatives of gouty patients even though they, the relatives, display no other clinical evidence of gout or gouty arthritis.

EXPERIMENTAL OBSERVATIONS

These data were obtained during the past five years from the investigation of one hundred and thirty six blood relatives of twenty seven patients with gout. A clinical diagnosis of gout (6, 7) was apparent in each of the patients. Twenty three of the twenty five males had x ray evidence of gouty arthritis. The two females had proved urate tophi. The concentration of serum urate (8) was determined four or more times. In all except one sample from one patient it was greater than 60 mgm per 100 cc. In the absence of renal insufficiency or leukemia an elevation of serum urate above 60 mgm constitutes important evidence in support of a diagnosis of gouty arthritis in patients with unexplained joint disease.

¹ This investigation was aided by a grant from the Corn Industries Research Foundation.

² The results of this investigation were presented at the meeting of the American Society for Clinical Investigation Atlantic City, New Jersey, May 2, 1938.

Exceptions to this may be noted in patients over 60.

No one of the one hundred and thirty six relatives appeared to be suffering from gout or gouty arthritis. Fifty-eight per cent of the group were males. The ages varied from 6 to 86. Most of them were in either the 3rd, 4th or 5th decade of life. The kinship to the gouty patients included parent, sibling, child, grandchild, niece, nephew and cousin. Two had rheumatoid arthritis, six degenerative joint disease, and two rheumatic heart disease. The remainder of the group were in apparent good health.

X rays of the feet were taken in one hundred and ten subjects. Changes considered to be consistent with gout were not observed in any. The concentration of serum urate was determined one or more times in each relative. One hundred and two had a concentration less than 60 mgm per 100 cc. In a few instances the determinations were repeated and the normal values were checked. The average for the one hundred and two subjects was 4.6 mgm. This is slightly higher than the average for a similar number of non gouty subjects (9). The concentration of serum non protein nitrogen was less than 35 mgm per 100 cc. in each member of this group.

The remaining thirty four relatives had a serum urate greater than 60 mgm per 100 cc. The serum values ranged from 6.1 to 10.8 mgm per 100 cc. (Table I). The average was 7.3 mgm. The determination was repeated in thirteen subjects one or more times within four years of the original observation and found elevated. The ages of these subjects ranged from 14 to 86. Eighty per cent were males. The genealogical trees of two families are given in Figures 1 and 2.

The serum nonprotein nitrogen concentration was less than 35 mgm per 100 cc. in each instance. Intravenous phenolsulphonphthalein and urine concentration tests were studied in a few. The tests were normal. Other causes of serum urate were excluded.

medical history and by physical examination. It is apparent that an elevated serum urate may be observed in members of gouty families who present no other evidence of gouty arthritis. It is concluded that such an elevation is intimately associated with the constitutional gouty diathesis and is not the result of renal disease.

It is of interest that, during the eighteen to thirty-six months that have elapsed since this communication was prepared for publication, three of the thirty-four have had one or more attacks of acute arthritis. Their ages at the time of the acute attacks were 40, 46, and 47, respectively. One or more joints of the feet were involved. X-ray changes or subcutaneous tophi were not demonstrable subsequently. A presumptive diagnosis of gout in these three subjects is justified probably on the basis of family history, elevated

TABLE I—Continued

Family	Relatives with a normal concentration of serum urate Number	Relatives with an increased concentration of serum urate		
		Age	Sex	Serum urate <i>mgm per 100 cc</i>
He	1	72	Male	7.7
McG	1			
Mor	2	54 63	Male Male	7.4 6.8
Mort		48	Male	6.3-9.5-7.1
Na	5	34	Male	7.3
Pe	7			
Re	5	24 29 57	Male Male Male	6.8-7.9 7.9 9.1
Ri	5	65	Male	6.1
Sa	1	20	Male	6.1-7.0
Sch	1	37	Female	6.2
St		58	Male	6.5-6.9
Ta	12	39 46 86	Male Male Male	6.4-6.7 9.6-10.0 6.4-6.2-6.3
Tran	5	28 14	Female Male	6.7 6.4-6.4
Tort	1			

TABLE I

Experimental observations on twenty-seven gouty families

Family	Relatives with a normal concentration of serum urate Number	Relatives with an increased concentration of serum urate		
		Age	Sex	Serum urate <i>mgm per 100 cc</i>
An	13	17 20 21 25 43	Male Male Female Female Female	6.3-6.9 7.7 6.2 10.7 7.0
Br		38	Male	8.0
Bu	5	17 20 37 42 47	Male Male Male Female Male	7.0 6.6 6.1 7.0 6.2-6.7
Caf	3			
Can	2			
Cent	3	37	Male	6.5
Char	3			
Chas	5			
Col	8	18	Male	9.4-6.6-8.8
Cora		40	Male	9.5-6.2-7.4
Cron	14	46	Male	6.6-6.1
Di		24	Male	7.4
Fa		37	Female	6.4

serum urate and an acute attack of arthritis. On the other hand, no one of the subjects who had a concentration of serum urate less than 6.0 mgm per 100 cc has had any acute attacks which suggested gouty arthritis. Obviously, it is hazardous to predict whether any more of the relatives will develop symptoms of arthritis in the future.

In addition to the blood relatives, the wives of five patients with gout, whose sons had an elevated serum urate, were interviewed. The concentration of serum urate was less than 5.0 mgm per 100 cc in each of them.

DISCUSSION

Twenty-five per cent of one hundred and thirty-six relatives of gouty patients showed an increase in serum urate above 6.0 mgm per 100 cc. It is believed that this is a manifestation of a familial

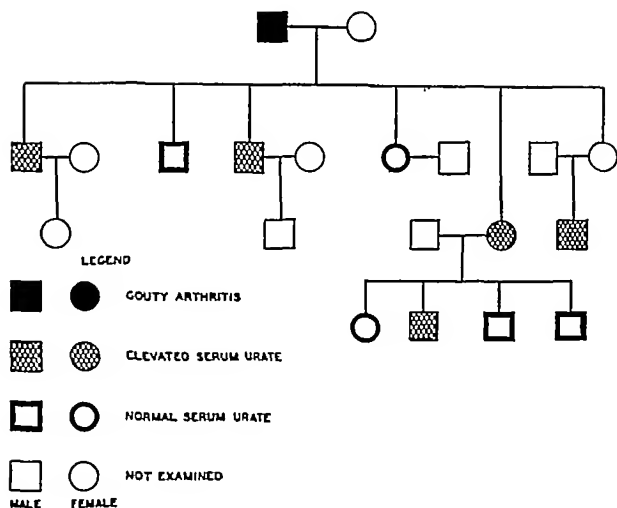


FIG. 1 GENEALOGIC TREE OF FAMILY BU

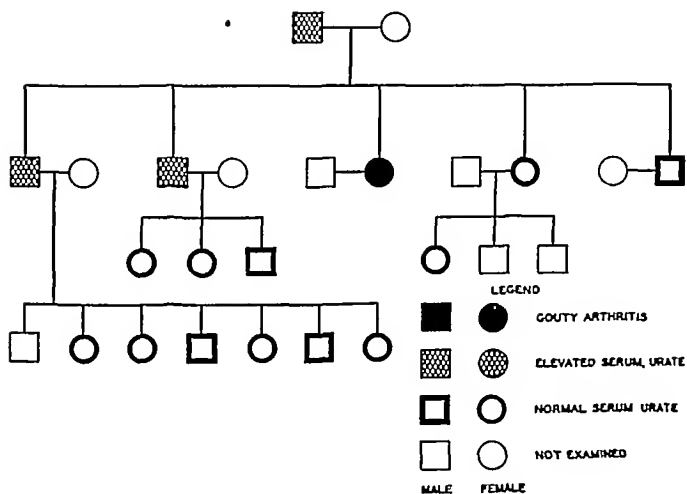


FIG. 2 GENEALOGIC TREE OF FAMILY TA

tendency If this assumption is correct, it is probable that an increased concentration is present at birth or shortly after, although the youngest age observed by us was 14 The fact that three subjects were older than 70 indicates that an elevated level in the body is compatible with good health and a reasonably long life The high incidence of males in the group with an elevated serum urate agrees precisely with the sex distribution of clinical gout

Little is known concerning the etiology of gout other than that it is a metabolic dyscrasia associated with an increased concentration of urate in the body It is the belief of the writer that an increased formation of urate is the most significant factor in the gouty diathesis (10) If this hypothesis is correct, cases of gouty arthritis may appear in gouty families regardless of mode of living or environment The finding of an increased concentration of serum urate in non-gouty relatives of gouty patients lends additional support to the hypothesis that gout is a defect of purine metabolism, this defect being one of increased formation of urate

SUMMARY

A study has been made of one hundred and thirty-six relatives of twenty-seven patients with gouty arthritis At the time of their first examination no one of the relatives had had any symptoms or x-ray evidence of acute or chronic gout The serum urate was normal in one hundred and

two of the group, the average was 4.6 mgm per 100 cc In the remaining thirty-four, the concentrations varied from 6.1 to 10.8 mgm per 100 cc, the average was 7.3 mgm per 100 cc Eighty-three per cent of these subjects were males It is concluded that an elevated serum urate, an essential component of the gouty diathesis, may be observed in symptom-free members of gouty families

The following persons assisted in this study Dr F S Coombs, Mrs E A. Gall, Mr W V Consolazio, and Mr L J Pecora.

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STUDIES ON PAIN A NEW METHOD FOR MEASURING PAIN THRESHOLD OBSERVATIONS ON SPATIAL SUMMATION OF PAIN

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The purpose of these studies has been to present a new method for measuring pain thresholds, together with experimental observations on the physiology of pain and on the effect of various chemical agents upon pain thresholds

Methods for estimating the intensity of the stimulus required to evoke a painful sensation in the skin may be classified under the headings mechanical, chemical, electrical, and thermal. The mechanical devices as used by von Frey (1) and by Eddy (2), involving production of pain by pressure, empirically correlated the amount of pressure and the pain threshold. The deformation and internal stresses in the tissues responsible for the sensation were not investigated and it may not be assumed that there is a simple proportionality between the pressure and the intensity of the sensory stimulus. The chemical methods of irritating the skin have not been thoroughly explored.

The thermal methods of producing pain are among the oldest and were introduced as a method of study by Goldscheider (3) in 1884. These methods, involving application of hot objects to the skin or immersion of a member in hot water, have added little of a quantitative nature to the study of pain sense. The only method of study for which the relation of the stimulus to its physiologic and sensory effects has been investigated is the electrical method of stimulating with Faradic current introduced by Helmholtz (4) and studied carefully by Martin (5). This method has been widely used, largely in the study of the action of analgesics by Martin and his co-workers and by Macht and his associates (6). Recently Hauck and Neuert (7) have used high frequency currents in the study of pain thresholds.

Valuable work has been done with the above methods but none of them combines the flexibility and precision necessary for some investigations. The use of thermal radiation for the study of pain

has many advantages, the most important of which are

- 1 The necessary apparatus is simple and easily constructed
- 2 The intensity of the stimulating agent can be precisely measured.
- 3 The sensory threshold to pain as a result of this stimulus is a sharply defined experience so that thresholds may be determined with accuracy higher than that of other methods
- 4 The method is flexible so that the time of exposure to the stimulus, the state of the skin, etc., can be varied at will
- 5 The stimulus can be used for large and small areas of skin even though the surface be irregular
- 6 The stimulus can be repeated in rapid succession without injury to the skin surface tested.

Radiation was first used to stimulate skin sensation by Alrutz (8) in 1897. In 1921 Sonne (9) focussed the rays of the sun onto the skin to produce pain. Neither of these authors was studying pain although Sonne showed that the white human could stand more penetrating radiation than non penetrating radiation. In 1934 Dallenbach (10) used radiation to produce pain in an investigation of adaptation.

The experiments of Oppel and Hardy (11) demonstrated that the radiation technique could be applied quantitatively to the study of temperature sense, and the methods devised by these authors have been adapted to the measurement of pain thresholds. The pain is produced by concentrating the radiant energy from a powerful source onto the skin. The sensation produced is sharp a 'bright pain' (12) and is to be distinguished from an ache or deep pain. Whereas our observations apply specifically to pain in the skin, it is probable that they have broader implications.

METHOD

The apparatus for measuring the pain threshold is shown schematically in Figure 1

The light from a 1000 watt lamp, *L*, was focussed by a condensing lens, *C*, through a fixed aperture onto the blackened forehead of the subject, *H*. The surface of the forehead to be tested was thoroughly blackened with India ink. This measure was taken to insure total absorption of the radiation, regardless of pigmentation of the skin, and to eliminate possible effects due to the penetration of the rays below the skin surface. The stimulus could thus be considered as purely thermal

The intensity of the radiation was controlled by means of a rheostat, *RH*. Immediately in front of the lamp was mounted an automatic shutter, *P*, which was arranged to allow the radiation to pass through to the subject for exactly 3 seconds. This time interval was so short that the heating of the skin was local and effects due to conduction at the edges of the aperture could be neglected. Thus, the temperature changes in the exposed area were assumed to be uniform. It was necessary that the time of stimulation be fixed precisely as the pain threshold depended upon this factor. In the present apparatus *P* was fixed to a heavy pendulum. The shutter, *S*, was operated manually, and allowed stimulation of the subjects when desired.

The method of making the measurement of pain threshold was as follows. The subject seated himself and placed his forehead in position. The aperture was arranged so that 35 cm.² of blackened skin could be exposed. After a minute or so the shutter, *S*, was lowered and the radiation allowed to fall on the skin for 3 seconds. The subject reported on his sensation. If no pain was experienced, the intensity of the light was increased and after 30 to 60 seconds the test was repeated. This procedure was followed until the subject just felt pain at

the end of the exposure. This threshold pain was easily recognizable even by untrained subjects. The sensation was that of heat finally "swelling" to a distinct, sharp stab of pain at the end. When this condition had been reached the radiometer, *R*, was placed in the aperture in place of the forehead of the subject and the intensity of the radiation measured in gm. cal./sec./cm.². This value was considered to be the minimum stimulus for pain and was shown to be proportional to any thermal changes taking place in the skin, whether the total change or rate of change of skin temperature or both be considered. The radiometer was calibrated by means of a radiation standard of the U S Bureau of Standards and also with an experimental black body

The time required to make a single observation was usually less than 2 minutes. The maximum variation from the mean was in all cases less than 12 per cent, and several measurements agreeing within 2 per cent were made to establish a threshold. Among the physical factors which were found to influence the threshold were those which tended to increase the rate of heat loss from the skin. Sweating was found to cause a great decrease in the effectiveness of this type of stimulus. Also strong drafts and especially cold rooms had to be avoided. Circumstances which affected the condition of the skin, such as sunburn, calluses, or skin lesions, changed the level of the threshold. As the skin temperature had some influence on the threshold, most tests were made on the forehead. The forehead temperature is constant ($34.0 \pm 0.5^\circ \text{C}$) over a wide range of external temperatures ($20\text{--}28^\circ \text{C}$), barring sweating from any cause.

RESULTS

The measurements of pain threshold on the 3 subjects over a period of a year are shown in

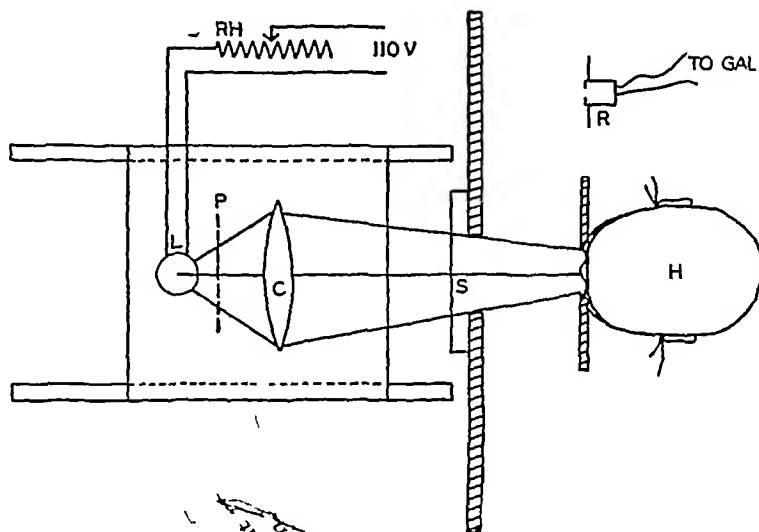


FIG 1 ARRANGEMENT OF APPARATUS FOR MEASURING PAIN THRESHOLDS

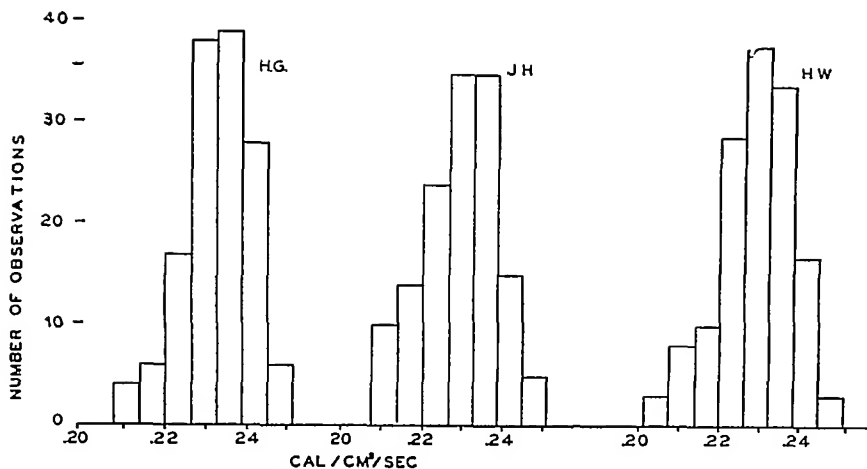


FIG. 2. FREQUENCY CHARTS OF PAIN THRESHOLD MEASUREMENTS FOR 3 SUBJECTS

Figure 2 Half the measurements on any subject were within ± 2 per cent. Further all 3 subjects had nearly the same thresholds, i.e., 0.229, 0.231, 0.233 gm cal/sec./cm² for H G W, J D H, and H G, respectively. Any single measurement could be expected to be within ± 5 per cent of this average value. Daily measurements of pain thresholds were made over a period of about a year. Before measuring pain thresholds, estimates were made by each subject concerning his or her general effectiveness as regards daily tasks and emotional state. These varied considerably from day to day but their effect on pain threshold was in every case less than 12 per cent, and there was no constant relationship between any mood variant and the pain threshold. There was no anxiety on the part of the subjects as regards the experimental procedure and the fact that the pain threshold remained independent of emotional state should not be taken to mean that the subjects reacted to the pain in the same way every time. Martin (5), using Faradic current as stimulus, observed a diurnal variation in pain threshold or irritability with a low point at 10 a.m. to 11 a.m. and a high point at 4 p.m. to 5 p.m. On the other hand, Macht (6) with the same method, could demonstrate no change at all during a 26-hour experiment. Our measurements, made at various times of the day between

9 a.m. and 7 p.m., showed no significant changes with time of day.

Except for procedures directly affecting the skin and chemical agents having analgesic action, we have demonstrated few factors affecting the pain threshold. Binding the head firmly with a bandage through which a window was cut for the threshold measurement was found to increase the average threshold by about 4 to 6 per cent. This rise though definite shows that an intense sensation due to a stimulus which is not painful affects the pain threshold to a minor degree. Gripping a bar as tightly as possible was found to raise the threshold in one subject 7 per cent and in another 15 per cent. As gripping the bar was slightly painful, a larger increase in threshold resulted than in the case of bandaging the head. An extremely loud and penetrating noise produced by striking a metal plate held just behind the subject's head caused a rise of 14 to 32 per cent in 2 subjects depending upon the duration of the stimulus. Continued striking for 30 seconds produced a rise of 25 to 30 per cent. The intensity of sound was so great as to be 'painful.' It would be expected that other factors, such as the excitement of a contest or an accident would have appreciable effects on the reaction to pain. This matter will be discussed in a subsequent communication.

The nature of the sensations resulting from irradiating the skin is of interest. In 1884 Goldscheider (3) claimed to have demonstrated that the sensation of pain or burning is not mediated by the end organs of heat. In order to make certain that in these experiments we were dealing with pain and not a strong sensation of heat, the following two experiments were performed.

In the first experiment the thresholds of pain and of heat were determined on 2 subjects. Then 18 grams of acetylsalicylic acid were given by mouth. The changes in thresholds for both heat and pain were followed for 4 hours (Figure 3). The pain threshold was raised 35 per cent, whereas the heat threshold was actually lowered 55 per cent. This differential effect of the drug clearly separates the two sensations. In the second experiment it was shown that the peripheral structures mediating pain and heat sensations are entirely separate. Pain thresholds were measured on the back of the left hand and on the forehead. A sphygmomanometer cuff was then wound around the left arm above the elbow and inflated

to a pressure of 200 mm of Hg. The pain thresholds on hand and forehead were followed for 35 minutes, the pressure in the cuff released, and thresholds followed until normal values were again obtained. Estimates of the amount of pain resulting from the manometer cuff were made, and the "6+ pain" just before release of the pressure represents an almost intolerable state. It was repeatedly demonstrated that after 35 minutes of ischemia, sensation in the hand had almost disappeared with the exception of pain. From this experiment it seems probable that pain and temperature are not served by the same peripheral apparatus.

Figure 4 demonstrates the course of the above experiment. The solid line represents the change in threshold of the ischemic hand, and the dashed line the change in threshold of the forehead. Since the pain threshold in the ischemic arm was elevated to the same degree as that in the forehead, one may conclude that the observed effect was due to pain resulting from the ischemia rather than from the ischemia itself. It has been men-

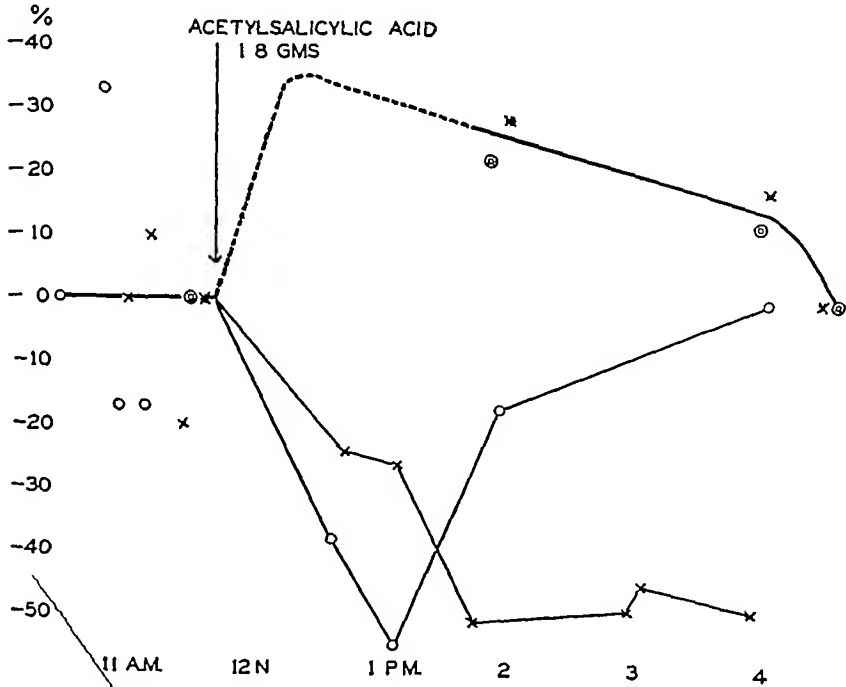


FIG 3. EFFECT OF ACETYSALICYLIC ACID UPON THE THRESHOLDS TO HEAT AND TO PAIN

Double circles and dotted crosses = pain, single circles and crosses = heat (Dashed portion taken from different experiment with same amount of agent.)

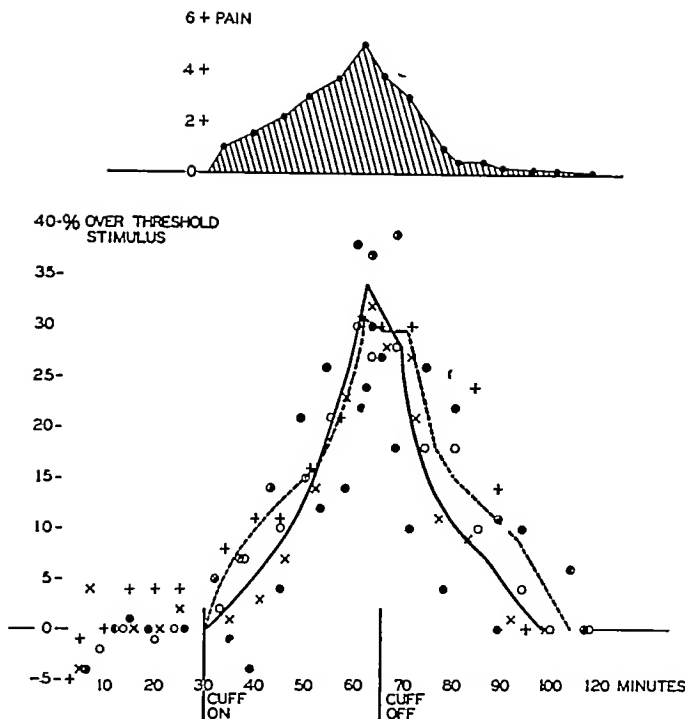


FIG. 4 THE EFFECT OF ARRESTING THE BLOOD FLOW TO THE LEFT ARM UPON THE PAIN THRESHOLD IN THE LEFT HAND (DASHED LINE) AND ON FOREHEAD (SOLID LINE)

Upper shaded area represents the subjective estimates of the pain caused by the ischemia in the arm.

tioned above that pain induced by methods other than those involving ischemia also raises the pain threshold

Furthermore, the above experiment indicates that pain occurring in one part of the body causes a rise in the pain threshold in the rest of the body. It also appears that the pain threshold rose with the increase in the intensity of pain from the cuff. The maximum height of this effect was between 30 and 40 per cent above the normal threshold. Also, as will be discussed in a later communication, various chemical agents raise the pain threshold (13).

Hardy and Oppel (14) demonstrated almost perfect spatial summation for heat and cold sen-

sations on the forehead. That is, as the area exposed to the radiation was increased, the strength of stimulus necessary to evoke sensation decreased almost in proportion. Application of this method to pain was made in an attempt to demonstrate spatial summation. The experiments on pain were carried out in a similar manner to those on temperature sense, except that smaller areas were stimulated. However, series of areas were used, with a ratio of extreme sizes of 400:1. The data for 2 subjects are shown in Table I.

From the smallest to the largest areas there was a slight decrease in the intensity of stimulus necessary to evoke pain. This may be due to slight spatial summation, or what is more likely, to the

TABLE I

Intensity of radiation required to evoke pain, with three seconds' exposure, for various sized areas of skin on the forehead

Area	Subject G	Subject H	Average
cm ²	gm cal /sec./cm ²	gm cal /sec./cm ²	gm cal /sec./cm ²
0 07	0 287 0 314	0 247 0 265	0 277
0 13	0 262 0 264 0 269	0 245 0 257 0 252	0 258
0 19	0 259 0 254 0 265	0 257 0 255 0 255	0 258
0 95	0 258	0 228	0 243
3 46	0 249	0 228	0 236
7 10	0 240	0 242	0 242
10 00	0 240	0 225	0 233
28 30	0 256	0 241 0 241	0 245

subject's underestimation of the pain threshold as a result of the strong sensation of heat which precedes the pain with the large areas. For the largest area, the heat stimulus was more than 400 times the threshold value, for the smallest area the sensation of heat was often not present at all. Thus, when large areas of the forehead were exposed to the radiation, the thermal sensation was so strong that experience warned that pain was imminent, and with the smallest areas in which no heat sense was present pain was felt only as a prick. It is necessary that the time of exposure be short (3 seconds or less) for this experiment in order to obtain the same heating rate for all sized areas. Figure 5 shows a comparison of the area stimulated and intensity of stimulus required to evoke the sensations of heat and pain. The comparison is made on the basis of the rate of rise of skin temperature. Other relations, such as total change in skin temperature and the rate of energy absorbed, are proportional to the stimulus measured in these terms. It is evident that there is little, if any, spatial summation of pain sensation of the type found with heat sense or cold sense.

The pain curve (Figure 5) shows the relationship between intensity of stimulus and area which must exist if there be no spatial summation. The

existence of this relationship, however, does not prove that there is no spatial summation for pain. That is, even though enlarging the exposed area did not reduce the intensity of stimulus necessary to evoke pain, spatial summation for stronger stimuli was not excluded. Thus, 0.230 gm cal/sec/cm² may represent both the threshold of excitation for the end organs and the sensory threshold of pain, and a lower stimulus would not be expected to evoke a pain regardless of the amount of spatial summation present. This would correspond to the finding of Hardy and Oppel (14) for temperature sense with large areas. (They observed that when 200 cm² of surface had been exposed they could not further decrease the threshold stimulus no matter how much larger area was irradiated.) There is subjectively a "bigger" pain with 28 cm² than with 0.07 cm² of exposed surface, although the intensity of pain did not seem to be different.

In order to test whether or not spatial summation exists with supraminimal stimuli and to apply threshold measurements for such a purpose, it was necessary to administer an analgesic agent (morphine) whose action can be assumed to be central rather than peripheral. If under such circumstances there were spatial summation for these supraminimal stimuli, the pain threshold would be raised by the morphine to a smaller degree in a large area than in a small area. In other words, if increasing the size of the area exposed had had the same effect as increasing the intensity of the radiation, the morphine would have raised the pain threshold less for the large area.

The following experiment was performed on 2 subjects. Threshold stimuli were measured for areas 0.3 cm² and 3.46 cm², the latter representing more than eleven-fold increase in area. This area ratio, on the basis of the summative effects found in temperature sense, would have been ample to show spatial summation of pain if it existed. After obtaining normal thresholds, morphine was administered to the subjects and the change in threshold in these two areas was followed for 6 hours. Since morphine acts centrally, it may be assumed that pain impulses from both areas were being evoked when the stimulus was above the normal threshold and that more pain impulses were arising from the larger area than from the smaller.

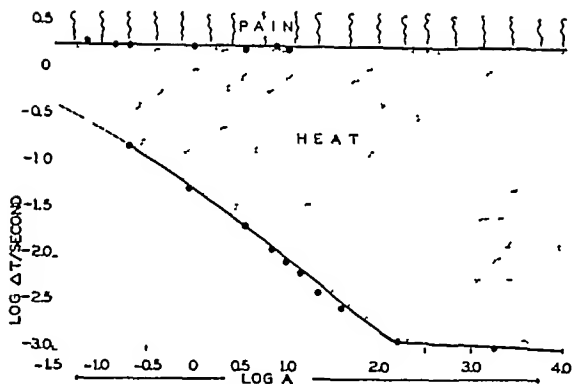


FIG. 5. RELATIONSHIP BETWEEN LOG INTENSITY OF THRESHOLD STIMULUS (RATE OF CHANGE OF SKIN TEMPERATURE IN DEGREES PER SECOND) AND THE LOG AREA STIMULATED

Upper curve = pain lower curve = heat.

The results of the experiment are shown in Figure 6, and it is seen that the morphine caused the same rise in threshold to pain in the two areas. Thus, one must conclude either that morphine acts to prevent spatial summation or that summation

for pain does not exist. In any case it has not been possible to demonstrate spatial summation for pain.

Lack of summation indicates that the central representation for superficial pain is different

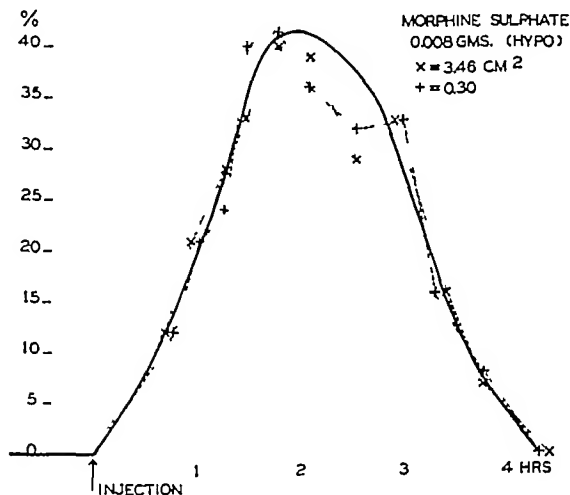


FIG. 6. EFFECT OF MORPHINE UPON THE THRESHOLD STIMULI FROM LARGE AND SMALL AREAS

from that of temperature and touch. Pain is increased in intensity only by increasing the intensity of the stimulus and not by association of larger numbers of impulses from various sources. Therefore, the evidence from both the peripheral and central components of the pain apparatus suggests that functionally pain is distinct from other sensations. These demonstrated differences imply a system of discrete neural units for pain sense. It is useful to consider pain as a unique esthetic experience with its own physiological properties and neural mechanisms.

The range of stimuli from the excitation threshold of most sensations up to the pain threshold is great. With sight the ratio is greater than 10^6 1, with temperature 10^3 1, and other senses have ratios lying in this range. The body, because of spatial summation, is therefore extremely sensitive to stimuli of these types. The ratio between the stimulus evoking threshold pain and that causing tissue destruction is about 2 to 1. Thus, $0.230 \text{ gm cal/sec/cm}^2$ will evoke a threshold pain in 3 seconds, in the same time $0.460 \text{ gm cal/sec/cm}^2$ will invariably produce blistering and cause an extremely intense pain. Obviously, a similarly intense pain would result from a relatively mild stimulus over a large area where there is spatial summation for pain, mitigating against the organism. Adequate provision has been made through pain to warn the organism of the approach of tissue damage without effecting a sensitiveness which would make life unbearable. It is nevertheless true that the individual is aware of multiple pains and that discomfort is increased according to the size of body area involved. For many obvious reasons a sunburn over the entire body surface will cause more distress than that over a 1 cm^2 area, even though the intensity of the pain in each case be the same. In this sense the distress of the organism must be considered as a function of both the intensity of the stimulus and the size of the area over which it is effective.

SUMMARY AND CONCLUSIONS

1 A quantitative method for measuring pain thresholds in the skin by thermal radiation has been described. The method has the general advantage of measuring a physical quantity which is

directly proportional to the changes occurring in the skin. The method has the further advantages of precision, simplicity of technique, rapidity of measurement, and the fact that the stimulus is innocuous upon repeated application except at high intensities. Further, any part of the skin surface may be studied and the size of the stimulated area varied at will.

2 Pain thresholds measured in this way did not vary consistently with time of day, with the general effectiveness, or the emotional state of the 3 subjects.

3 Individual threshold measurements for 3 subjects were 0.229, 0.231, and $0.233 \text{ gm cal/sec/cm}^2$ and all measurements were found to be within ± 12 per cent of their respective average values. The standard deviation for a single measurement was calculated to be ± 2 per cent.

4 Intense pain in any part of the body raised the pain threshold in the skin in other parts as much as 35 per cent.

5 The senses of pain and heat, which were always stimulated together, were shown to be separate sensations through the action of acetylsalicylic acid. This drug lowered the heat threshold and raised the pain threshold.

6 The peripheral structures responsible for pain sense were distinguished from those of temperature and touch by demonstrating that occluding the blood for 25 minutes did not directly affect the pain threshold in the ischemic hand, whereas other sensations could hardly be elicited.

7 Pain sense was found to have no spatial summation in the sense that the pain threshold for many end organs was no lower than that for a few. This was observed to be the case for minimal stimuli and for supraminimal stimuli after morphine administration.

8 The intensity of radiation which produced blistering in 3 seconds was observed to be twice that necessary for the bare perception of pain.

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STUDIES ON PAIN MEASUREMENT OF THE EFFECT OF MORPHINE CODEINE AND OTHER OPIATES ON THE PAIN THRESHOLD AND AN ANALYSIS OF THEIR RELATION TO THE PAIN EXPERIENCE

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Though valuable observations have been made of the action of analgesic agents these have been based in the main on animal experimentation and clinical impression (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11). No adequate method for assaying their effects on the pain threshold in man has been available. However, since the prime purpose of an analgesic drug concerns its action in man it is desirable to measure accurately its effect on man's pain threshold. For such measurement a suitable method has now been developed (12). Also, it is important to define precisely other analgesic effects. It has thus been possible to evaluate the therapeutic effectiveness of the opiates and to make inferences concerning the nature of the pain experience in man.

METHOD

Quantitative measurements of the pain threshold were made by exposing 35 cm.² of skin surface for 3 seconds to thermal radiation. The intensity of radiation which barely evoked pain was denoted as the pain threshold. In this way the normal pain threshold level was established to ± 2 per cent by making observations at 5-minute intervals until a constant threshold was obtained. This usually required four observations. After the control measurements an analgesic agent was administered and observations of the pain threshold were made at 10-minute intervals until the threshold had returned to the control level that is, until all pain threshold raising action had ceased. The height of the pain threshold raising effect was expressed in per cent elevation above the control level. The protocols were distributed so that the threshold of each subject was measured by a colleague who in turn was unfamiliar with the change in his own threshold. Occasionally observers not participants in the experiment made threshold readings. Thus three independent protocols were made, no individual knowing how much his own threshold had been altered. Occasionally sterile salt was introduced into one of the syringes so that it was known to all that one of the subjects had not received an analgesic drug although which one had been so treated was not revealed until the end of the experiment. All agents were administered intramuscularly.

With each pain threshold reading the subject made a concise statement of his psychological state. In the 10-minute intervals between readings the subjects sat comfortably and engaged in reading writing or conversation. Sleep was not permitted for reasons to be discussed later and if drowsiness became difficult to manage the subjects walked about the laboratory. During long experiments food was taken, but not until it had generally been conceded that the peak of action had been passed. The 3 subjects then consumed about the same amount of milk and bread. This usually took no longer than 15 minutes, after which the readings continued as before.

Morphine in various amounts was administered to each subject on 18 different occasions and codeine on 15. Thus, a total of 54 morphine and 45 codeine experiments was performed on 3 subjects. The time interval between such experiments was seldom shorter than 1 week and usually 2 weeks or more. Thus the possibility of acquiring a tolerance to morphine and codeine was minimal. Furthermore, it was demonstrated by a repetition of standard quantities about once a month that no tolerance to morphine had been developed. At the end of the study the threshold raising effects of 15 mgm. of morphine and of 60 mgm. of codeine were the same as at the beginning of the study. In addition, experiments were made with other opium derivatives, namely dihydromorphine (Dilaudid), methylhydromorphine¹ (Metopon), pantopium hydrochloride (Pantopon,² Roche) and combinations of scopolamine and morphine.

SUBJECTS

The subjects studied were the 3 authors of the paper. They represented both sexes and different body types. One of the males was a tall, linear person, the other shorter, more muscular and 'thick set.' The woman was tall and well developed. All weighed about the same, *ie* 65 kgm. The 3 individuals resembled each other in the possession of more than average energy and curiosity but differed from each other temperamentally in life orientation and in interests. They were conscientious witnesses and as might be expected in

¹ Courtesy of Dr Howard L. Andrews U S Public Health Service.

terested and willing. Since it was necessary to make these determinations on persons who were suffering no pain other than that induced by the experiment, it was unreasonable to expect any but those most concerned to expose themselves to this inconvenience.

OBSERVATIONS

Threshold In the manner described above, the pain threshold was determined daily and before each experiment. It was found, as has been discussed in detail elsewhere, that the individual's pain threshold varied but little from day to day and also that the pain threshold of the 3 subjects differed but slightly.

During the first series of experiments the subjects were free of all pain other than that induced by the test procedure. Freedom from pain before and during the period of pain threshold measurement is important. It will be shown in the second part of this communication that pain, *per se*, raises the pain threshold and that pre- or co-existent pain, either spontaneous or induced experimentally, seriously alters the threshold-raising action of morphine and codeine. Menstruation, which introduced no factor of discomfort in the female subject, did not change her pain threshold.

The subject's reaction to the experimental procedure was recognized as having potentially important effects upon the observations. In order to evaluate this effect, several experiments were done in which one subject received a placebo. A typical experiment is shown in Figure 1. It can be seen that the procedure itself was neutral so far as threshold-raising effect was concerned.

SERIES I

Morphine sulphate Observations The results of experiments with 8 different quantities of morphine from 0.1 mgm to 30 mgm are shown in Figure 2. The threshold to pain was observed to rise within 10 minutes following injection.

The height and duration of the threshold-raising effect increased with the amount administered. Whether the amount of the agent was large or small the threshold began to rise after the same interval of time but continued to rise at different rates until the peak effect for the particular amount had been reached.

20-4%

15-

10-

5-

0-

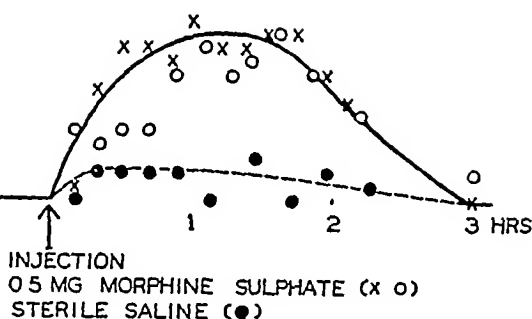


FIG 1 THE PAIN THRESHOLD-RAISING EFFECT OF 0.0005 GRAM OF MORPHINE SULPHATE ON TWO SUBJECTS

A third subject received sterile saline. Points X and O represent the pain threshold elevation after 0.0005 gram of morphine sulphate, ●, the effect of sterile saline. The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect.

As can be seen in Figure 2, when the morphine was given in quantities from 0.5 mgm to 15 mgm, the peak of action was reached almost at the same time, namely, in approximately 90 minutes. With 30 mgm, however, the peak effect was not reached until 150 minutes after administration. The duration of action from control threshold back to control threshold varied from 3 hours in the case of 0.5 mgm amounts to somewhat over 7 hours in the case of 30 mgm amounts. The 0.1 mgm amount of morphine sulphate was apparently without any threshold-raising action.

Comment The time-action curves of Figure 2 can be analyzed, as regards the amount of agent given, in three ways:

- (1) the maximum height of the pain threshold-raising effect, (2) the length of the period of effectiveness, and (3) the total threshold-raising action of the agent.

The maximum height of the threshold-raising action was obtained from the highest part of each time-action curve.

The length of the effective period was taken to be the elapsed time between the injection and the return of the threshold to the pre-injection level.

The total threshold-raising action of the agent was computed by multiplying the duration in hours of effect by the average per cent above the control

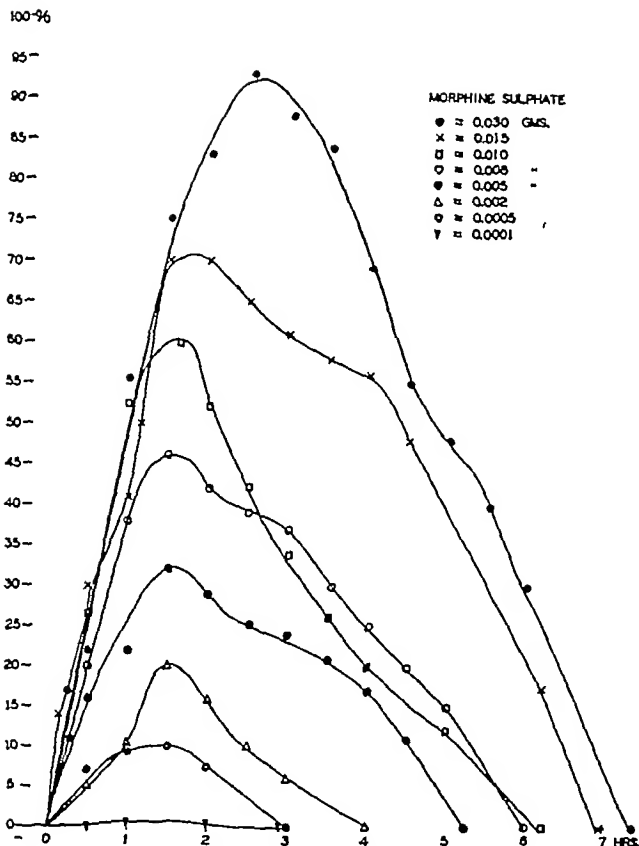


FIG. 2. TIME ACTION CURVES FOR MORPHINE SULPHATE

Pain threshold elevation after morphine sulphate in quantities of 0.0001 gram to 0.030 gram. The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect. The O indicates the time of injection of the morphine sulphate. Each point represents the average of the threshold levels for 3 subjects.

level. The latter figure was derived by taking the average of all the individual percentages represented by points on the curve of Figure 2. This is represented by the area under each time-action curve.

It will be seen (Figure 3) that the maximum analgesic effect in terms of quantity is a straight line function from 0.5 mgm. to 15 mgm. For

amounts greater than 15 mgm the increase in threshold raising effect with quantity becomes progressively less. Thus, doubling the amount of agent *et*, from 15 mgm to 30 mgm., increases the threshold-raising effect less than 20 per cent. The curve in Figure 3 may be considered in three parts (1) The abrupt change in the curve between 0.1 mgm. and 0.5 mgm. suggests that the minimal

MAXIMUM THRESHOLD RAISING EFFECT

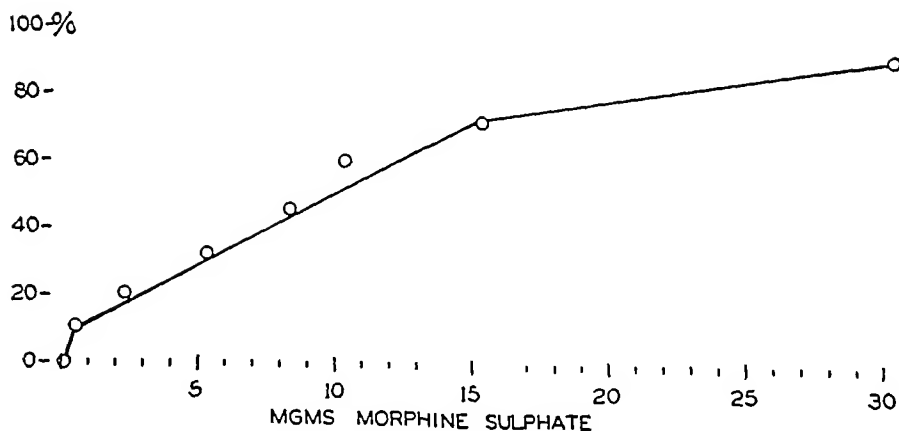


FIG. 3 THE MAXIMUM PAIN THRESHOLD-RAISING EFFECT OF MORPHINE SULPHATE FOR QUANTITIES FROM 0.0001 GRAM TO 0.030 GRAM

The ordinate = per cent elevation of pain threshold over the control level as zero The abscissa = quantity of morphine sulphate administered.

effective amount of morphine is approximately 0.5 mgm (0.007 mgm per kgm body weight). It is to be observed that this is less than 1/100 of the minimal effective dose reported by Eddy (6) and his associates (10) who used the cat as a test object. (2) The portion of the curve between 0.5 mgm and 15 mgm shows the threshold-raising effect to be in direct proportion to amount. (3) The latter portion of the curve (beyond 15 mgm) suggests a condition analogous to a chemical saturation, that is, the effect is not increased by increased concentration of one of the reacting agents. On this basis the "saturation" quantity of morphine sulphate, or the smallest amount with which the highest threshold-raising effect is attained, is close to 30 mgm. The "saturation" level, or the highest threshold-raising effect of which the drug is capable, is 100 per cent above the control threshold. This is in the range of tissue injury and blisters were regularly produced without pain in these experiments. Should increasingly larger quantities be administered, further threshold-raising effect, probably with unconsciousness, could be anticipated. Such amounts, however, were beyond the pharmacological range and the scope of this study.

The duration of threshold-raising effect increases with the amount given from 0.1 mgm to 30 mgm (Figure 4). However, the rate of increase with amount becomes progressively smaller

so that doubling the quantity from 15 mgm to 30 mgm causes only 5 per cent increase in the length of the effective period. This means that 30 mgm was rendered ineffective almost in the same length of time as 15 mgm so that the rate of essential elimination must proceed more rapidly with the larger amounts. Again, there is to be observed an abrupt change in the direction of the curve between 0.1 gram and 0.5 mgm, indicating a minimal effective dose between these amounts.

The relation of total threshold-raising action to amount is shown in Figure 5. There was a direct proportion between effect and amount up to 15 mgm. Doubling this amount, however, increased the total action only 25 per cent.

The time-action curves for all amounts studied were of a simple type, that is, the level of the threshold-raising effect rose with time until a maximum was reached and then descended relatively smoothly to the control level. The highest threshold-raising effects for amounts from 0.5 mgm to 15 mgm occurred at the same time. The fact that 30 mgm had its maximum threshold-raising effect 1 hour later than the lesser amounts indicates inhibition of the effective absorption².

² The words effective absorption are meant to include all the processes occurring between injection and the production of analgesia. Essential elimination is used to mean the rate at which the agent is rendered ineffective as an analgesic.

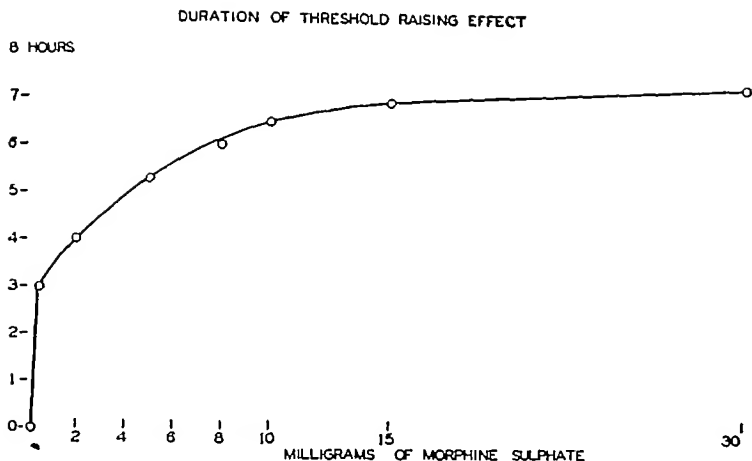


FIG. 4 THE RELATION BETWEEN DURATION OF THRESHOLD-RAISING EFFECT (ORDINATE) AND THE QUANTITY OF MORPHINE SULPHATE ADMINISTERED (ABSCISSA)

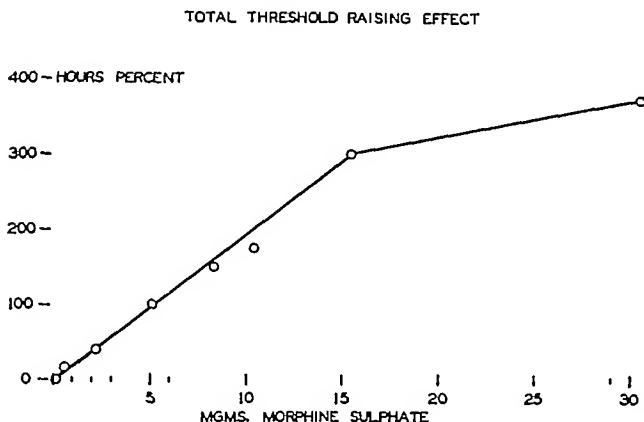


FIG. 5 THE RELATION BETWEEN THE TOTAL THRESHOLD-RAISING EFFECT AND THE QUANTITY OF MORPHINE SULPHATE ADMINISTERED

The ordinate was computed by multiplying the average per cent rise in pain threshold by the hours of duration of effect resulting from a given quantity of morphine sulphate. The abscissa represents quantity of morphine administered.

after the larger quantity. It would appear further that the essential elimination has actually been accelerated in this instance since the effective period of threshold-raising was only 5 per cent longer than that for one-half this amount, or 15 mgm.

Morphine sulphate. Psychological effects, observations. In addition to the rise in pain threshold, the administration of morphine resulted in relaxation, freedom from anxiety, lethargy, apathy, and difficulty in mentation. Outstanding was the freedom from anxiety and feeling of contentment. The pain threshold-raising action was not closely related in time to these psychological changes, the latter effects outlasting the threshold-raising action by many hours.

After the administration of morphine in amounts from 5 mgm to 30 mgm, the following effects were observed in varying degrees, depending on the quantity given. Within 3 to 4 minutes after the injection the subjects became aware of feelings of muscle relaxation about the extremities, the neck, and the back. This was soon followed, approximately 5 minutes after injection, by changes in mood or attitude. Thus, the subjects before injection were alert and preoccupied with technical problems associated with the progress of the experiment and its successful culmination. After injection all seemed to be going well. Time between readings, which formerly seemed long and tiresome, became short and pleasurable. Loquacity was evident within a half hour after the injection. Conversation was agreeable and steady without push. Anxieties or dilemmas not concerned with the experiment also seemed to be readily soluble, and anticipation of events immediately to follow the experiment was free from conflict. This change in attitude was maintained for the next 2 to 3 hours.

This freedom from anxiety or "contentment" was associated with a constructive attitude toward the problems at hand. Fears, inhibitions, and doubts were reduced. Discriminations and decisions became easier. The mood changes were also described as "good humor," "euphoria," "pleasurable relaxation," and "exhilaration."

After about 30 minutes, attention, retention, and concentration became more difficult as did clear, logical or continuous thinking. Such difficulties in mentation continued for 3 to 4 hours.

Commonly, freedom from anxiety was associated with and followed by apathy. There was an increasing indifference to situations and decisions, with little concern about either difficulties or opportunities. As a manifestation of apathy, the attitude toward vomiting experienced with the 30 mgm quantities was instructive. The vomiting was repeated and violent and was associated with no more emotional reaction than would ordinarily accompany rinsing the mouth or swallowing.

Lethargy outlasted the above effects and was present often for as long as 24 hours. Toward the end of this time it ceased to be accompanied by a mood which made acceptance or indifference possible. Apparently, in reaction to a decreased effectiveness, the subjects experienced annoyance, impatience, and resentment. Such states became manifest sometimes as soon as 4 hours after injection and persisted for 24 to 72 hours. The extent of the over-irritability was conditioned by the subject's awareness of his defects and by the stress to which he was exposed. When, during this period following the experiment, he was in a relatively neutral environment with few decisions of importance confronting him the mood reaction was of a less distressing nature. When, however, he was confronted with major decisions and either failed to recognize or make allowances for his lowered effectiveness, this period of 24 to 72 hours after the morphine injections was one of increasing tension and depression. Moreover, when the post-morphine period came just before menstruation, depression and tension were sometimes accentuated and prolonged.

Other morphine effects. "Full-headedness" or headache was repeatedly experienced by 2 of the subjects about 5 minutes after the injection. These head sensations were gone within a half-hour, sometimes recurring 12 hours later. Feelings of faintness, and "light-headedness" had their onset and termination within a half-hour of the injection. Unsteadiness of gait was noted within a half-hour of the injection and was short-lived.

Itching of the nose was a complaint of 2 of the subjects. The symptom had its onset between 1 and 2 hours after the injection, and lasted from 2 to 4 hours. Itching elsewhere (pubic region) occasionally occurred.

Dimming of vision associated with reduction in

the size of the pupil was observed within a half-hour of the injection. Slight constriction of pupils without dimming of vision could still be seen 10 to 14 hours after the injection

Nausea had its onset characteristically within 10 minutes after the injection and with smaller amounts disappeared within a half-hour. After large quantities of morphine it recurred in "waves" for 2 or 3 hours after the injection and with 30 mgm. persisted for 8 hours with vomiting which was repeated and vigorous

The administration of 30 mgm of morphine sulphate produced, in addition to the changes described above, a state akin to prostration. Pallor, loss of initiative, nausea, vomiting, sweating, weakness, and unsteadiness of gait were the dominant features. Three mgm of dihydromorphi-

none hydrochloride ("Dilaudid") had a similar effect

Codeine phosphate Observations Codeine phosphate was injected intramuscularly in amounts from 15 mgm to 120 mgm. The time-action curves for this agent are shown in Figure 6. As in the case of morphine sulphate, the maximum threshold raising effect was reached at approximately the same time for all the aforementioned quantities, i.e. about 90 minutes. The duration of action varied from 3 hours with doses of 15 mgm to somewhat more than 5 hours after 120 mgm. The minimal effective quantity of codeine was not determined.

Comment The maximum pain threshold raising effect of this agent was proportional to the amount administered up to 60 mgm (Figure 7)

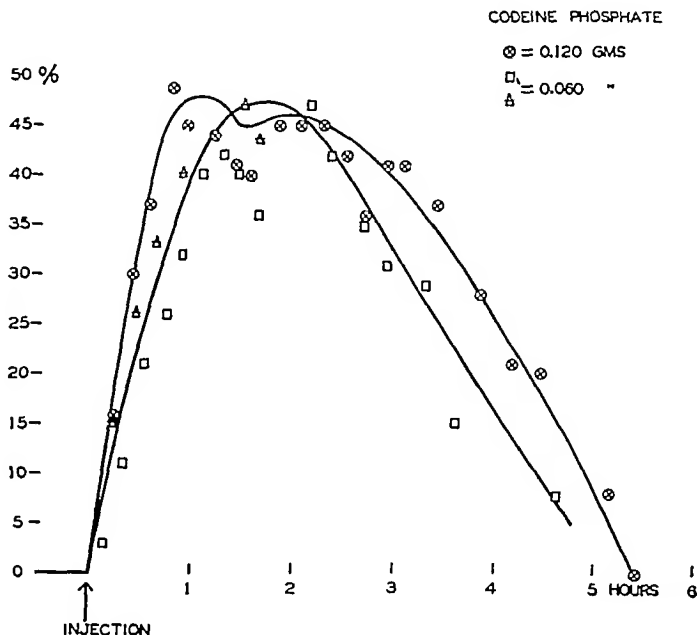


FIG. 6. PAIN THRESHOLD ELEVATION AFTER QUANTITIES OF 0.060 GRAM AND 0.120 GRAM OF CODEINE PHOSPHATE

The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect. Each point represents the average of the threshold level in 3 subjects.

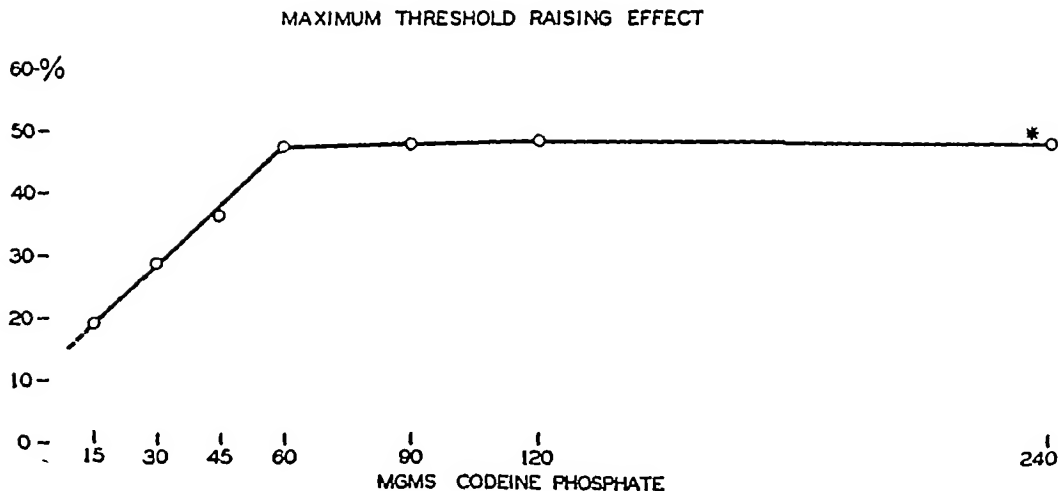


FIG 7 THE MAXIMUM THRESHOLD-RAISING EFFECT OF CODEINE PHOSPHATE FOR QUANTITIES FROM 0.015 GRAM TO 0.240 GRAM *

The ordinate = per cent elevation of pain threshold over the control level as zero The abscissa = quantity of codeine phosphate administered

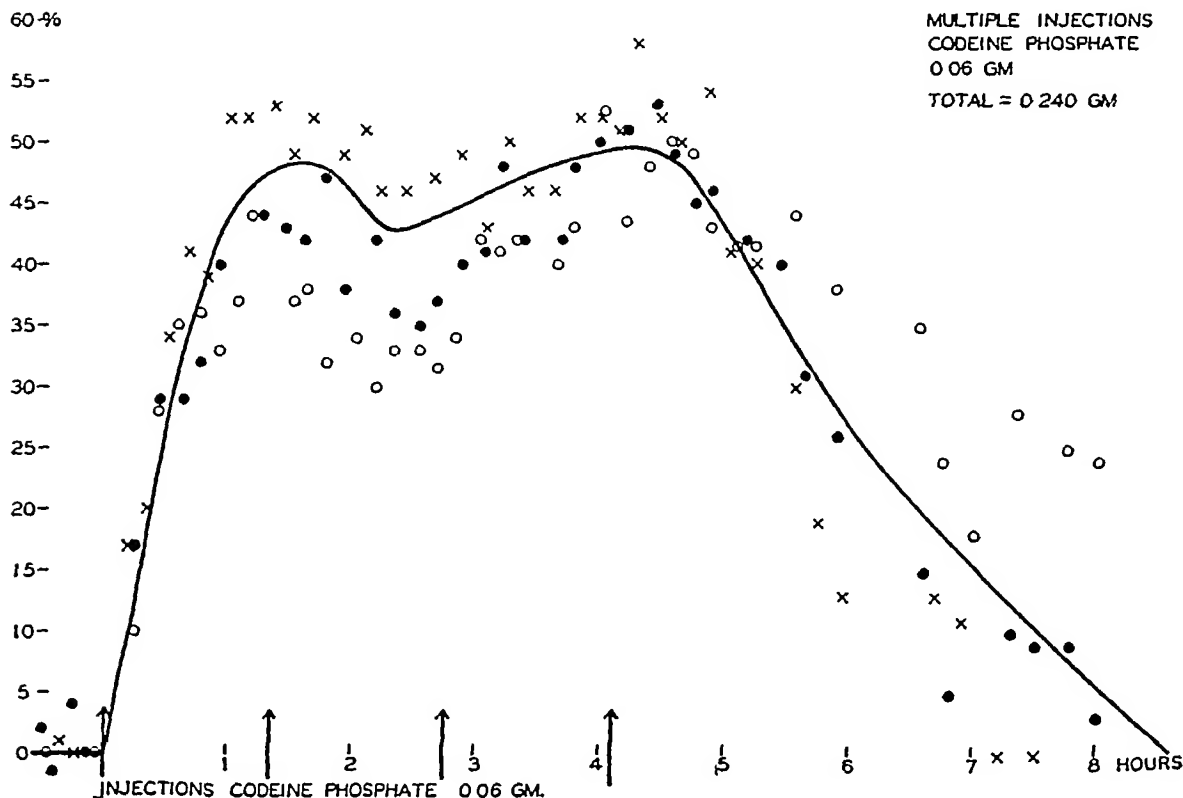


FIG 8 THE EFFECT ON THE PAIN THRESHOLD OF REPEATED INJECTIONS OF CODEINE PHOSPHATE, 0.060 GRAM, AT 80-MINUTE INTERVALS

The ordinate = per cent elevation of threshold above the control level as zero The abscissa = duration of effect. The points ●, X, O represent the individual threshold levels in 3 subjects

* 0.240 gram given in four injections of 60 mgm. each, 80 minutes apart

(0.92 mgm. per kgm. body weight) resulting in a rise of 45 per cent to 50 per cent above the normal threshold. Administration of double this amount, or 120 mgm., caused no further rise in threshold. Therefore, 60 mgm. may be considered the saturation quantity for codeine and a 50 per cent rise in threshold above the control may be considered its saturation level.

To test the concept of "saturation" level and quantity, the following questions were posed: After the administration of 60 mgm. of codeine phosphate, when the pain threshold had been raised to the established maximum effect of approximately 50 per cent above the control level, would a second injection of 60 mgm. raise the level further? Would the action of the second 60 mgm. start from this high level, or would it have no influence on the pain threshold other than to maintain it at the level which had been reached by the first 60 mgm.?

Observation. Figure 8 shows that despite three attempts to raise the threshold above the previously attained maximum of about 50 per cent through the addition of 60 mgm. of codeine at 80-

minute intervals, no further elevation was observed. The threshold-raising effect was not increased beyond that produced by the first 60 mgm. injected. The threshold returned to the control level 4 hours after the last injection.

Comment. The above experiment demonstrates the validity of this concept of a "saturation" quantity and level, and shows that the only effect of additional amounts as regards threshold-raising is to prolong the action. As in the case of morphine, increasingly larger quantities ultimately could be anticipated to have further threshold-raising effect but again such amounts would be beyond the pharmacological range.

The total threshold-raising action of codeine, shown in Figure 9, increased proportionally up to 60 mgm. Thereafter, increasing the quantity increased the total action far less. Moreover, vomiting and prostration were experienced with 120 mgm.

Figure 10 shows the relationship between the duration of action and quantity of agent. Although the data are not so regular as they are for morphine, the relationship between these factors

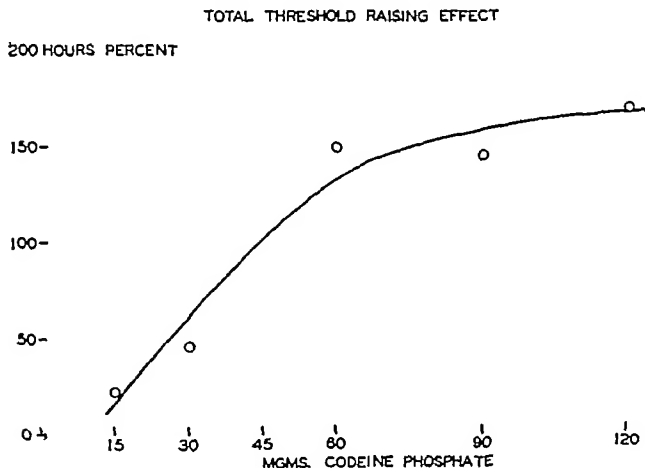


FIG. 9 THE RELATION BETWEEN THE TOTAL THRESHOLD-RAISING EFFECT AND THE QUANTITY OF CODEINE PHOSPHATE ADMINISTERED

The ordinate was computed by multiplying the average per cent rise in pain threshold by the hours of duration of effect resulting from a given quantity of codeine phosphate. The abscissa represents the quantity of codeine administered.

DURATION OF THRESHOLD RAISING EFFECT

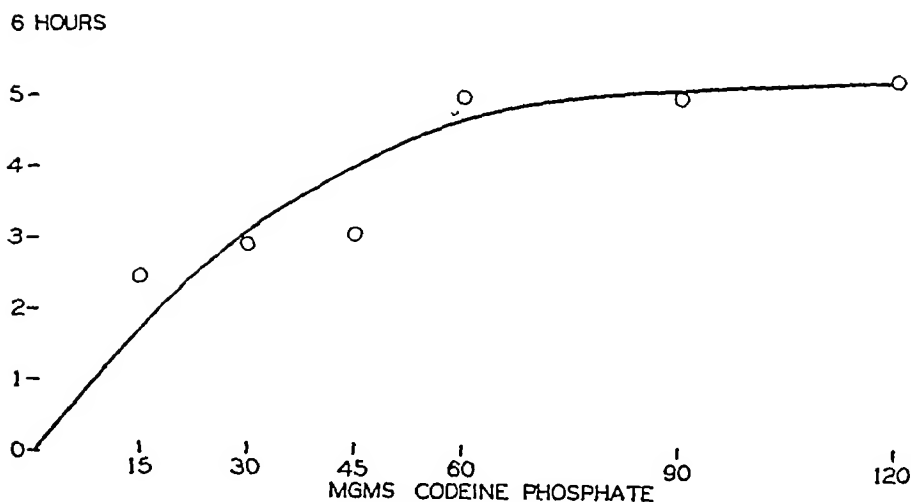


FIG 10 THE RELATION BETWEEN DURATION OF THRESHOLD-RAISING EFFECT (ORDINATE) AND THE QUANTITY OF CODEINE PHOSPHATE ADMINISTERED (ABSCISSA)

is similar. For codeine the "saturation" quantity is approximately 60 mgm.

Inspection of the time-action curves for morphine and codeine reveal (1) The height of threshold-raising action which can be obtained with codeine is limited to about one-half that of morphine. (2) The time-action curve of analgesia for morphine sulphate revealed that essential elimination increased at a constant rate with quantities up to 15 mgm. However, with larger quantities of the agent there was acceleration of the rate so that duration of effect with 15 to 30 mgm of morphine was about the same. The time-action curve of threshold-raising for codeine phosphate revealed essential elimination at a constant rate up to 60 mgm. With larger amounts within the range here studied, there was acceleration of the rate, as in the case of morphine. (3) The psychological effects produced by morphine and codeine are qualitatively similar, although morphine is capable of producing greater effects than codeine.

Eddy (6) and his co-workers (10), on the basis of most painstaking studies, have compared the action of various opiates with one another upon cats. Thus morphine was said to have ten times greater analgesic action than codeine. Such a statement unfortunately does not indicate the difference in the degree of threshold-raising that can be induced by the two agents with different

amounts as assayed here. For example, in man 2 mgm of morphine raised the pain threshold 20 per cent, whereas it required 15 mgm of codeine to raise the threshold a similar degree. Again, 60 mgm of codeine were necessary to raise the threshold 50 per cent, whereas only 8 mgm of morphine were required. On the other hand, 15 mgm of morphine raised the threshold 75 per cent but no amount of codeine within the pharmacological range can raise the threshold to a degree equal to 15 mgm of morphine. It may be that the discrepancy between these results and those of Eddy is centered about the difference in sensitivity of the methods of assay.

Several other well known derivatives of opium and combinations were tested to determine if they possessed more effective pain threshold-raising qualities or other advantages over morphine or codeine.

Dihydromorphinone hydrochloride ("Dilaudid" (Bilhuber Knoll)) Three mgm of this agent (7) were given intramuscularly to 2 subjects. The psychological effects were pronounced and the pain threshold-raising action dramatic. At the peak of its action the threshold was raised somewhat over 100 per cent (Figure 11). This effect was approximately the same as that observed after the administration of 30 mgm of morphine.

Lethargy was pronounced and there was repeated vomiting. The psychological effects were

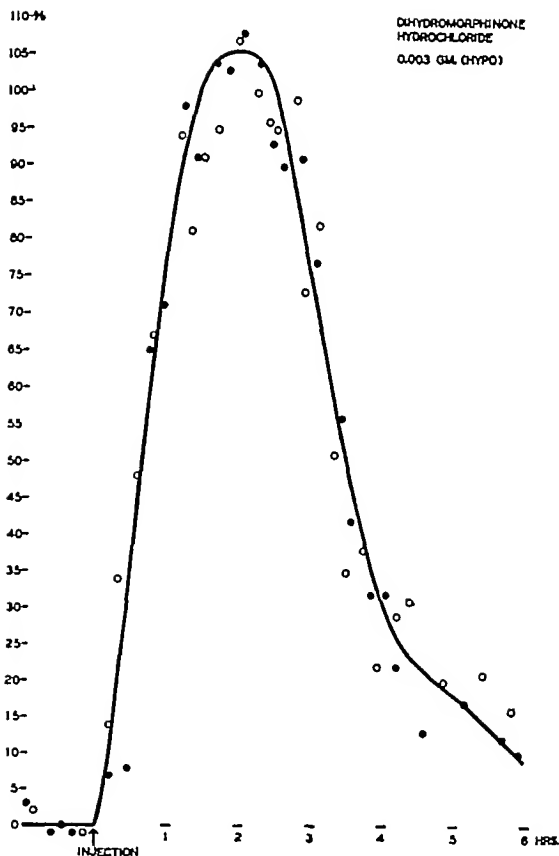


FIG. 11 THE PAIN THRESHOLD ELEVATION RESULTING FROM THE ADMINISTRATION OF 0.003 GRAM DIHYDROMORPHINONE HYDROCHLORIDE ("DILAUID")

The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect. The points O and ● represent the threshold levels in 2 subjects.

little different from those experienced after 30 mgm of morphine sulphate.

Pantopium hydrochloride ('*Pantopon*' (Roche)) Twenty mgm. of *Pantopon*, given intramuscularly, raised the pain threshold about 35 per cent and the duration of the effect was 5 hours (Figure 12). Many of the common psy-

chological and physiological effects of morphine were encountered. The threshold raising action of 20 mgm of *pantopium* was equivalent to about 8 mgm of morphine sulphate.

Methyldihydromorphinone (*Metopon*) The injection of 66 mgm of *methyldihydromorphinone* (11) had a threshold raising action which

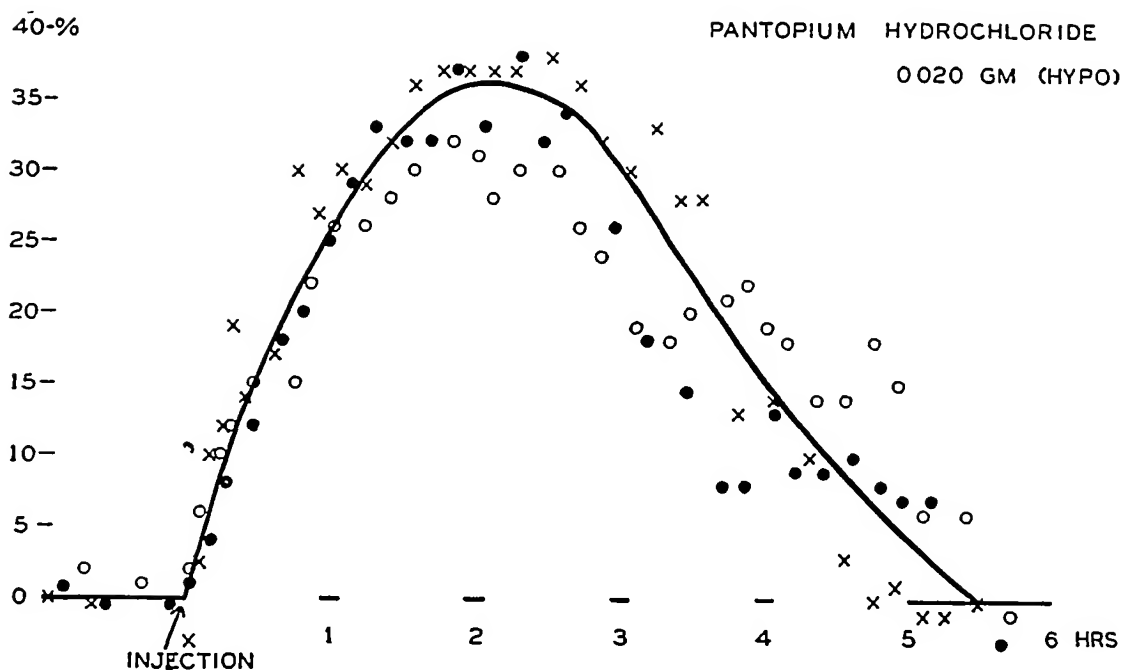


FIG 12 THE PAIN THRESHOLD ELEVATION RESULTING FROM THE ADMINISTRATION OF 0.020 GRAM PANTOPIUM HYDROCHLORIDE ("PANTOPON," ROCHE)

The ordinate = per cent elevation of threshold above the control level as zero The abscissa = duration of effect The points O, X, ● represent the individual threshold levels in 3 subjects

was comparable to approximately 30 mgm of morphine sulphate. The peak effect was attained in about 90 minutes and remained at a level of approximately 90 per cent above control threshold for $1\frac{1}{2}$ hours, after which the threshold gradually lowered, reaching the control level in about 7 hours after administration (Figure 13).

Vomiting began 2 hours after injection and occurred intermittently for the next 2 hours. Constriction of the pupils was noted within 10 minutes of the injection and persisted for at least 10 hours. The symptoms of prostration, as described with 30 mgm of morphine, were present.

Psychological effects were first manifested within 10 minutes of the time of injection. They began with feelings of "light-headedness," relaxation, and fullness in the head. The freedom from anxiety and feelings of contentment bordering on euphoria, characteristic of morphine, soon followed. Time sense was shortened. The mood alteration persisted for about 2 hours, after which followed lethargy and a state bordering on prostration. As demonstrated by the ability to retain numerals, concentration and retention were disturbed. Four hours after the injection there was

still lethargy and indifference to intermittent vomiting. The subjects were unable to differentiate these effects from those which were experienced with 30 mgm of morphine sulphate or with 3 mgm of dihydromorphinone hydrochloride ("Dilaudid"). However, the sequelae of methyldehydromorphinone were of shorter duration, and 24 hours after the injection no after-effects were noted.

Morphine with scopolamine The combination of morphine sulphate and scopolamine hydrochloride (in amounts of 8 mgm and 0.4 mgm, respectively) was injected intramuscularly. The pain threshold-raising effect of morphine was not increased nor was the duration of action prolonged as a result of the addition of scopolamine. Indeed, the pain threshold-raising effect was less than was obtained with an amount of 8 mgm of morphine sulphate alone. All the usual physiological and psychological effects of morphine were present and the following appeared in excess: dry mouth, unsteadiness of gait, difficulty in accommodation, dry smarting eyes, difficulty in mentation, and lethargy. The combination may have assets as regards the induction of sleep or relaxa-

tion but its threshold raising action is not superior to that of morphine alone.

Comment The inferences from these experiments concerning the assay of threshold raising effects of opium derivatives might be criticized on the basis that they represent the action upon only one type of pain, namely superficial pain with its

burning, tingling, prickling, or "bright" quality and that they may not be valid for deep pain of the aching or cramp variety. Such a criticism is unjustified since the results obtained from deep pain induced by a balloon that distended the duodenum were not unlike those described above. The similarity between the effect of drugs on these

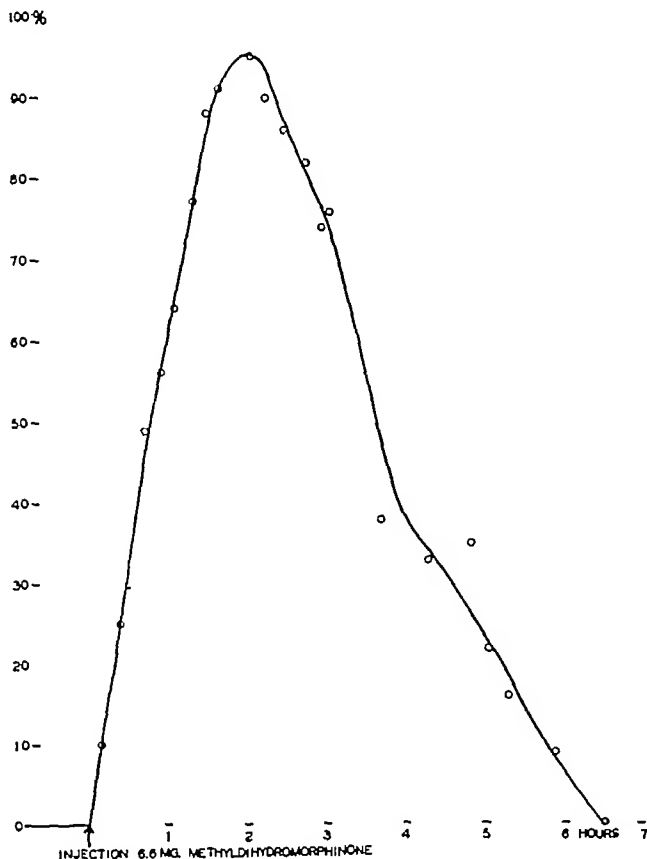


FIG. 13 THE PAIN THRESHOLD ELEVATION RESULTING FROM THE ADMINISTRATION OF 6.6 Mgm METHYLDIHYDROMORPHINONE (METOPON)

The ordinate == per cent elevation of pain threshold over the control level as zero. The abscissa == duration of effect. Each point represents the average of the threshold levels in 3 subjects.

two types of pain indicates that the results described would hold equally well for all types of pain

SERIES II

The aim of the first series of experiments was to demonstrate the threshold-raising action of morphine and its more important derivatives. The experiments were done with as few complications as the situation permitted, and pain and sleep were especially avoided. In the following experiments prolonged pain was introduced as a variable since in this way the action of morphine could be appraised more nearly in terms of its common therapeutic use.

Method In the manner described above the pain threshold was measured in the 3 subjects. To introduce a uniform stimulus of pain the following method was adopted. A sphygmomanometer cuff was applied over the upper arm and inflated to 200 mm. Hg pressure. It was left in this position for 40 minutes. The subjects contracted the muscles of the arm slightly and about the same amount. A gradually increasing, deep, aching pain resulted from such ischemia which began about 12 minutes after circulatory occlusion and continued for the remainder of the period, becoming progressively worse. The pain was described by the subject in terms of intensity from 1 to 6+. Six + and 8+ were considered intolerable pain. When the cuff was removed there followed 3 minutes of intense tingling and "pins-and-needles" sensations in the arm and hand. This terminated the painful experience.

To obviate the inference that the threshold-raising effect was due to the metabolic by-products of such circulatory occlusion, pain was produced by two other methods. The first of these produced pain by submersion of an extremity in ice water (3° to 5° C.) for as long as the pain persisted, then withdrawing and experiencing the pain which followed such submersion. One extremity after the other was immersed in this manner for a total of 40 minutes. The pain was intense and almost continuous.

The second method consisted of swallowing a catheter to which was attached a balloon which was distended with water when it reached the duodenum. The balloon was distended until a considerable pain resulted, which was then maintained for 40 minutes.

The third method was to compress the trapezius and biceps muscles by screw clamps and by manual compression until intense, deep, aching pain resulted. It was necessary to readjust the clamps from time to time. By means of such readjustments and manual compression an intense pain was maintained for 40 minutes. The pain induced by all of the above four methods had qualitatively similar effect on the threshold-raising action of morphine. The methods differed from each other in the in-

tensity of the pain which they induced, and in the quantitative effect on the morphine action.

After the control readings, which preceded the morphine injection, the painful procedure was begun (1) 46 minutes before injection, (2) 1 minute after the injection, (3) 50 minutes after the injection, (4) 120 minutes after the injection. Pain-threshold readings were made every 10 minutes throughout the subsequent 6 to 7 hours. The results were as follows:

Observations Morphine sulphate Pain induced early during the threshold-raising action of morphine and codeine altered the effect and the duration of the action. The longer the interval between the injection of morphine and the induced pain, the less effect was there upon the threshold-raising action of the agent. On the other hand, if the pain was induced just before or just after the injection of morphine the effect upon the subsequent threshold-raising action was dramatic.

In Figure 14 is represented graphically this effect of the introduction of pain on the pain threshold-raising properties of morphine sulphate. Pain was produced in four experiments by a cuff wound around the arm as described above. In the fifth experiment, pain was produced as follows. In subject H G W by muscle clamping, in subject J D H and H G by ice water, and in subject H G by duodenal distention. The heavy black line represents the pain threshold-raising effect of 15 mgm of morphine sulphate in 3 individuals without pain. By contrast, it is shown that a pain of approximately 40 minutes' duration, introduced immediately before injection, (curve 5), reduced the pain threshold-raising properties of the drug to an almost negligible amount, i.e., to average maximum effects of 7 to 12 per cent in 6 individual experiments, if introduced at the same time as the injection, the effect was shortened as indicated in the curve 4. The curve 3 shows the effect when pain was introduced 50 minutes after injection, and the curve 2 shows the effect when pain was introduced 2 hours after injection of morphine, when the threshold-raising action of the agent was at its peak. Pain reduced the intensity and duration of the previously described psychological effects.

Comment It may be seen that pain introduced immediately before or after the morphine injection had greater effect on its pain threshold-raising action than that introduced later after the pain threshold had been raised.

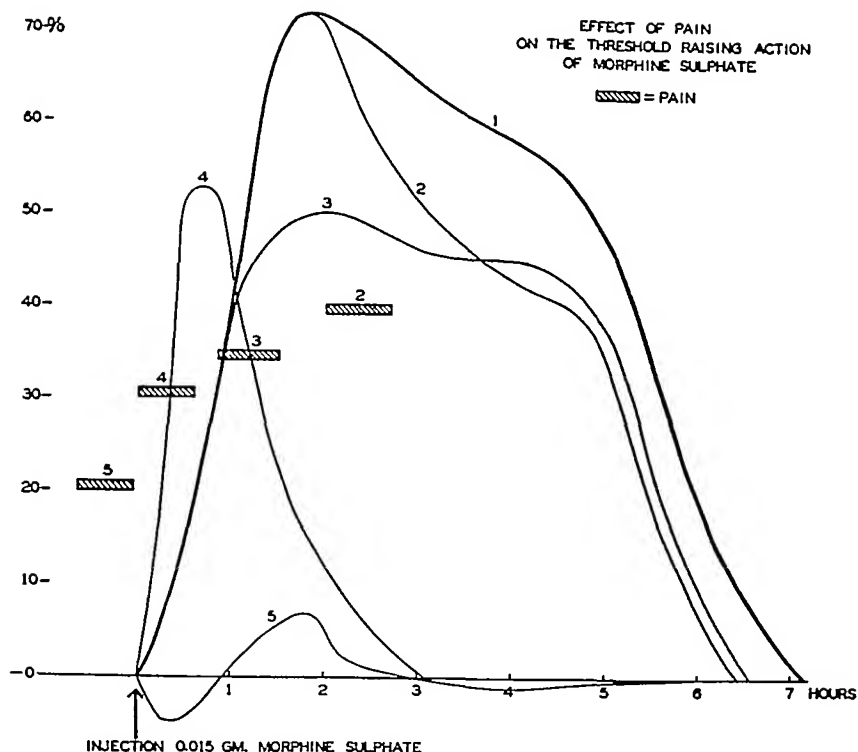


FIG. 14 THE EFFECT OF SUSTAINED PAIN (40 MINUTES) ON THE PAIN THRESHOLD-RAISING PROPERTIES OF MORPHINE SULPHATE (0.015 GRAM)

The heavy black line, 1, represents the pain threshold raising effects of 0.015 gram of morphine sulphate in 3 subjects without pain. The lighter lines and blocks represent the following: 2, Pain 120 minutes after the injection; 3, Pain 50 minutes after the morphine injection; 4, Pain immediately following morphine injection (after 1 minute); 5, Pain preceding and ending 6 minutes before the morphine sulphate injection. The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect. Each curve represents the average of the threshold levels in 3 subjects.

The reduced effect of the pain on the time-action curve when introduced after the threshold had been raised could not have been due to the fact that the subjects at this time perceived the pain to a lesser degree. Subject H G reported a 6+ pain from the manometer cuff inflated 120 minutes after injection, whereas H G W and J D H reported only $\frac{1}{2}$ to 1+ pain. The effect of the procedure upon the time-action curve was exactly the same in all three instances.

The time action curves of morphine shown in

Series I revealed two characteristics: (1) a period during which the morphine is being absorbed by the central neuronal pain mechanisms resulting in gradual elevation of the pain threshold; (2) the gradual elimination of threshold raising action. As the same painful procedure is more effective in reducing the threshold raising action the sooner it occurs in relation to the time of morphine injection, one may infer that the essential absorption is affected more than the essential elimination. In other words if the morphine has had

tunity to exert its influence upon nerve cells, the pain changes the time-action curve far less

Observations Codeine phosphate Pain had a similar impeding effect upon the pain threshold-raising action of codeine phosphate. The above described (cuff method) experience of pain was introduced shortly after the injection of 120 mgm of codeine phosphate. The threshold-raising effect of this quantity of the agent, as shown in Series I of this communication, was slightly over 50 per cent at its peak, with the threshold maintained near the 50 per cent level for about 2 hours, and gradually returning to the control threshold in about 6 hours after administration. After pain, the threshold had returned to the control level in from 2 to 3 hours (Figure 15). The application of a cuff to the other arm in 1 subject for a second 40-minute period immediately following the first

had no further detectable effect on the pain threshold-raising action of codeine. As in the case of morphine, pain reduced the intensity and duration of the characteristic psychological effects.

Comment It is to be noted in Figure 15 that, with the introduction of pain 9 minutes after the injection of codeine, the threshold-raising effect is probably the result of the induced pain rather than the codeine since, as has been shown before, pain has such an effect upon the pain threshold. The initial effect of the codeine during this hour, therefore, cannot be determined directly from this experiment. However, the subsequent threshold-raising effect was curtailed, as seen in the swift descent of the curve back to threshold level in 3 to 4 hours.

Morphine sulphate and codeine phosphate reacted similarly to the occurrence of pain during

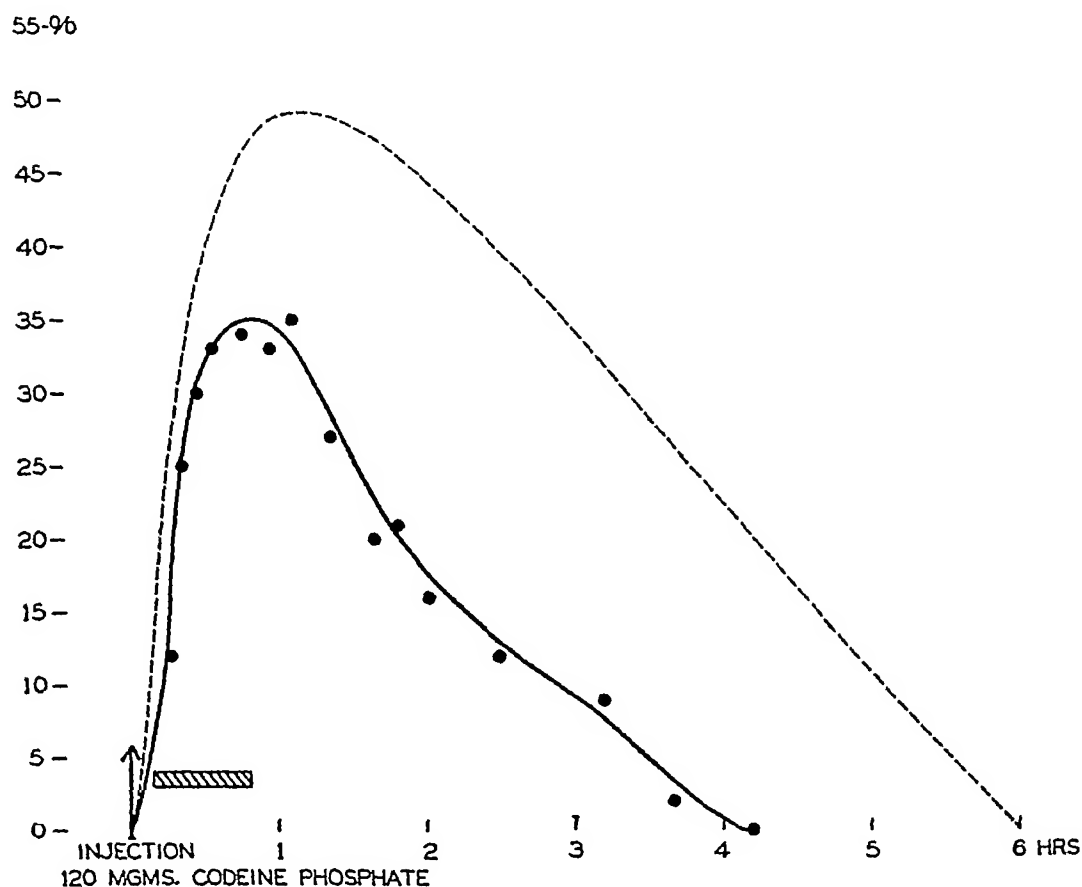



FIG 15 THE EFFECT OF SUSTAINED PAIN (40 MINUTES) ON THE PAIN THRESHOLD-RAISING PROPERTIES OF CODEINE PHOSPHATE

The ordinate = per cent elevation of pain threshold over the control level as zero. The abscissa = duration of effect.  = sustained pain (40 minutes)

their cycle of action. It appears that there is a mutual antagonism between pain and the derivatives of opium. That is to say, if the pain precedes the introduction of the agent, the analgesic action of the latter is limited or obliterated whereas, if pain is introduced after the threshold-raising effect has reached its peak, i.e., about 90 minutes after the injection, the effect of the pain is less evident.

Further analysis of pain as antagonist of morphine and codeine analgesia Since the experience of pain is associated with increased sympathetic activity, and presumably increased epinephrine output (13) it was considered desirable to appraise the action of epinephrine upon the threshold raising action of morphine. It is appreciated that epinephrine liberation would be but one of many effects of pain, but it is one that lends itself to experiment.

Observation Three subjects in two experiments received 10 mgm of morphine in the first experiment and 15 mgm in the second experiment. Two hours before the administration of morphine 1 cc. of 1:1000 epinephrine ('Adrenalin,' Parke Davis) was injected subcutaneously in one experiment and intravenously in the other. The latter was given as 300 cc of a 1:300,000 solution of epinephrine during 1 hour. Although blood pressure and pulse effects of the epinephrine had disappeared at the time of morphine injection the subjects still felt 'tense and excited.'

The effect on the threshold raising action of 15 mgm of morphine is seen in Figure 16. The well-defined threshold raising action of 15 mgm of morphine was completely obliterated in 2 of the 3 subjects. In the third subject, the threshold instead of being raised about 70 per cent by 15 mgm of morphine, was raised only 25 per cent. This occurred in the heaviest of the 3 subjects in whom the adrenalin had the least effect as regards feelings of tension and excitement. The psychological effects of the morphine were also reduced in intensity and duration. Similar effects were noted in the experiment after 10 mgm. of morphine.

Comment It is likely that the action of epinephrine in offsetting the morphine effects was on the central nervous system since even traces of circulating adrenalin show themselves in pulse and blood pressure alterations. In these subjects

at the time of morphine injection no such effects were observable. On the other hand, effects arising from central stimulation, namely feelings of tension, exhilaration and excitement were present. It is conceivable, therefore, that there had been some change in the state of the central pain mechanism as a result of the previous epinephrine injection making it refractory to the threshold-raising action of morphine.

The influence of pain and epinephrine on the pain threshold-raising action of morphine and codeine was not specific in the sense that analgesia alone was reduced or obliterated. Indeed all of the detectable morphine effects were less pronounced and of shorter duration. However, the threshold raising properties of morphine and codeine were profoundly disturbed by pre- or co-existing pain and more so than that action responsible for the altered emotional state. Such effects as freedom from anxiety, contentment, and relaxation, though less pronounced were still clearly evident. This differential effect made it appear as if pain had selected one function of morphine and spared the others.

Such central actions of epinephrine, or agents like it, are not without analogy since sympathomimetic agents are known to reduce the effect of anesthetics (14, 15).

The nature of the pain experience and its bearing on the action of morphine and its derivatives The data thus far considered present an apparent contradiction: (a) the analgesic action of morphine and codeine was reduced or obliterated by pain, (b) morphine and codeine undoubtedly reduce the distress experienced during pain. These seemingly contradictory observations can nonetheless be reconciled.

To begin with consideration of the data necessitates a formulation of pain which will include the two aspects of the pain experience.

1 *Pain perception* The perception of the sensation of pain is to be differentiated from the reaction pattern to pain. Pain is a sensation that differs from all others having its own neural apparatus and physiological properties and recognizable because of its unique esthetic qualities.

2 *The reaction pattern of pain* The reaction pattern of the organism to the sensation of pain usually follows promptly the perception of pain.

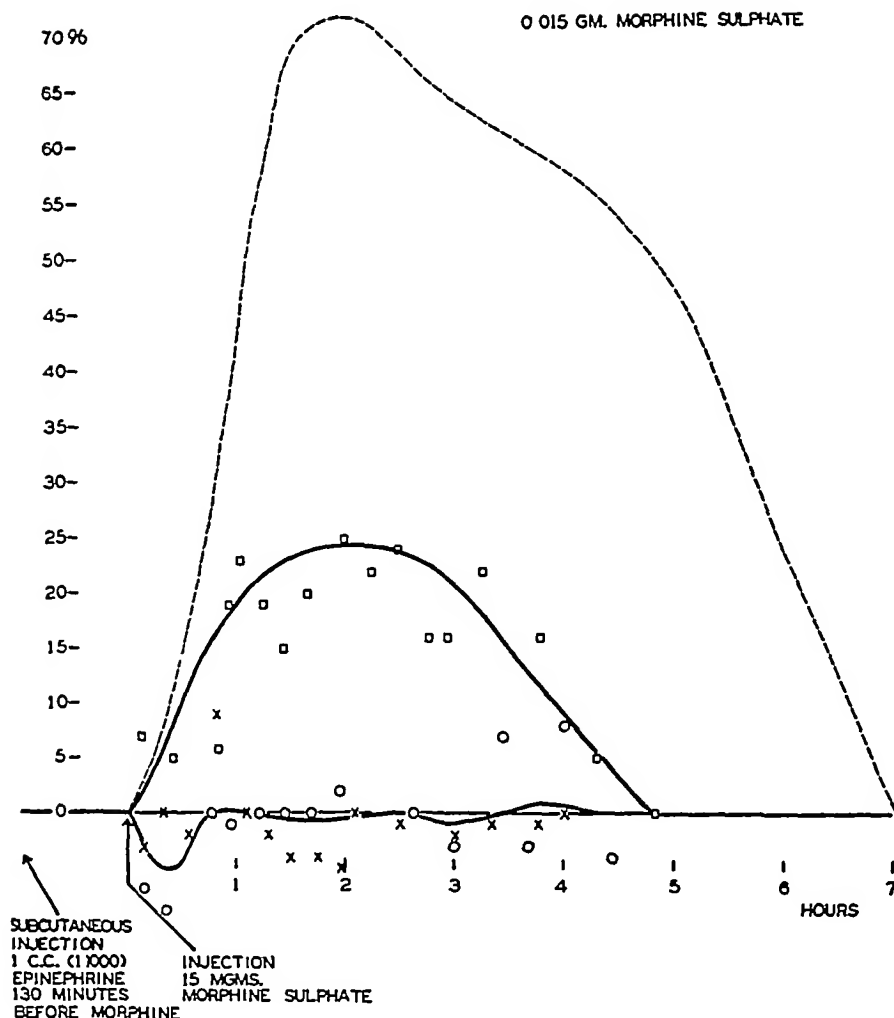


FIG 16 THE EFFECT OF THE ADMINISTRATION OF EPINEPHRINE ON THE PAIN THRESHOLD-RAISING ACTION OF MORPHINE SULPHATE

Dashed line = effect of 0.015 gram of morphine sulphate in the absence of epinephrine. Solid lines = effect of 0.015 gram of morphine sulphate after epinephrine administration. The points X, O, □ represent the individual threshold levels in 3 subjects

It has many components, including emotional, smooth muscle, gland and skeletal muscle expressions. The reaction pattern is, however, independent of perception and may be dissociated from it. It may, moreover, be modified or even obliterated.

It is the second of these aspects of the pain experience which will be further considered here. A pattern of withdrawal, flight, fight, and anxiety has become closely attached to pain perception. The perception of heat, sound, or cold resembles pain in that they are sensations which have asso-

ciated with them a pattern of response. However, the pattern of reaction to pain is unlike the pattern of reaction to sound, heat or cold in that it is more stereotyped, more predictable, and thus less readily recognized as capable of being dissociated from perception. However, there is a genuine distinction between perception and the accompanying reaction pattern. Such distinction is apparent and is easy to comprehend when the response is not stereotyped, as for example the responses to light, touch, heat, cold, and smell. But when, as in the case of pain, there is an inborn

as well as a further elaborated stereotypy developed from experience the contrast between perception and reaction may not be so apparent

The effect upon the subjects' attitude toward pain when it was induced for experimental purposes, without morphine, is relevant. Outstanding facts concerning the pain in these experiments were A realization that it could be terminated immediately if desired, satisfaction in the completion of a certain period of pain for the sake of the successful outcome of the experiment and appreciation that tissue injury was minimal and that there would be no untoward sequelae from the pain experienced In other words, the attitude toward the pain was one of relative relaxation, interest, and satisfaction because of its experimental significance. There was complete freedom from anxiety Hence, very painful procedures could be tolerated with relative impunity After such prolonged periods of pain the subjects experienced lethargy, drowsiness, or relaxation that could readily have been followed by sleep

Other instances of similar dissociation of pain perception from the pattern of reaction to pain are seen in the following

- 1 The indifference to injury sustained during the excitement of games or combat
- 2 The absence of reactions to pain when, during the influence of suggestion, hypnosis or catalepsy, tissue is injured
- 3 The apathy or 'quietism' that accompanies tissue damage during autosuggestion and during religious and mystical practices
- 4 The indifference to tissue damage during sexual excitement.
- 5 The indifference to pain often witnessed during parturition in women who are confident in their physician and desirous of bearing a child

Such a dissociation of perception of, and reaction to, pain may be of many varieties with varying degrees of completeness Further the individual may or may not be aware of such dissociation For example, in our experimental procedure there was complete awareness of pain as a sensation although it was largely dissociated from reaction In contrast, during combat one may even be unaware of pain sensation as well as free of pain reaction There may be more or less denial of pain reaction with repression of vocali-

zation or flight, yet with many other visceral reactions to pain, including syncope. In other words, the reaction to pain may be dissociated from perception in many ways Distinction between the two is essential to an understanding of the pain experience.

Let us consider now the contribution made by opium and its derivatives to our understanding of these dual aspects of the pain experience Morphine, for example, has at least two actions (1) it impedes to a greater or lesser degree the perception of pain (2) it detaches the perception of pain from the fight flight anxiety so closely attached to it. It is this aspect of the morphine experience which is variously described, but which is the common denominator of almost everyone's experience during its action Thus, freedom from anxiety, contentment, apathy and indifference about time are common experiences Not only anxiety but pain itself no longer arouses feelings of distress The pain sensation is perceived and is recognized as pain with no difficulty, but as a result of the morphine action there does not follow thereon the old and well-established reaction pattern to pain. In short, the opiates dissociate pain perception from the reaction pattern.

Experimental data present other instances of such dissociation of perception from reaction (16) For example, a dog after considerable training developed a fixed response to a strong negatively conditioned stimulus a metronome beat, which had never been accompanied by food. After repeated presentation it elicited zero salivary response in contrast to a bell which had always been associated with food and which called forth 200+ cm of saliva. Five minutes after the strong negatively conditioned stimulus (the metronome) had been presented, the dog was exposed to the stimulus of a flashing electric light which had heretofore been constantly accompanied by food but which was a weak positively conditioned stimulus When presented by itself, it elicited a moderate salivary reaction 150+ cm. of saliva. Now however, when offered so soon (3 minutes) after the strong negative stimulus the salivary reaction pattern to the light was abolished and there was no saliva. Four minutes later the light elicited again 142 cm. of saliva. There is no doubt that the stimulus was perceived by the dog because with the flashing light +

mal turned its head and body toward it. In other words, although the animal had perceived the light, the perception was dissociated from the reaction which that stimulus usually evoked.

It is possible that in an analogous manner, after morphine administration, the subject exposed to pain perceives the pain sensation, although the pain has been dissociated from the reaction pattern which that stimulus evokes. The organism is therefore in a state that allows perception, perhaps almost as complete as that before the administration of morphine, but which prevents the pattern of reaction from assuming its completed form. It is for this reason, possibly, that patients exposed to severe colic pain often state that the pain after morphine is not gone but that it seems to have no significance.

Further evidence that the threshold-raising effect of morphine can be dissociated from other morphine effects is offered by the cat. It has been shown by Eddy and his associates that the opiates produce a predictable threshold-raising action with regard to pain. Yet, it is common experience that, after opiates, the cat's behavior differs dramatically from that of man. Indeed, frenzy, rather than contentment, is a common feature in cats.

Cushny (5) mentions that, while a constant pain in a patient is alleviated by morphine, a suddenly introduced painful stimulus causes the patient to be aware of the new pain as though he had had no morphine. This discrepancy may be understood as follows. As a result of the morphine his changed emotional state would make him react indifferently to his constant pain. Yet, since morphine may not appreciably raise the pain threshold in a subject having prolonged pain, he might be keenly aware of any new painful stimulus.

Opiates as therapeutic agents The therapeutic action of opiates may be considered under three categories: (1) the threshold-raising action, (2) the property of dissociating pain perception from reaction in such a way as to free pain of its implications, and (3) the property of inducing lethargy and sleep.

1 Threshold-raising action When opiates are administered 90 minutes before the introduction of pain, their threshold-raising action is consider-

able and is probably an important part of their therapeutic usefulness. This is exemplified by the analgesic effect of morphine when administered before surgical operative procedures. When thus given, the "saturation" quantity of codeine phosphate is 60 mgm and optimal pain threshold-raising effects with morphine sulphate are attained with 15 to 30 mgm. After larger quantities, vomiting and other distressing symptoms often result. During severe pain of long duration, the threshold-raising action of the opiates is reduced and may even be obliterated. Under these circumstances their therapeutic value is chiefly through effects on the reaction pattern to pain and sleep-inducing effects.

2 Alteration of the pattern of reaction to pain A major function of the opiates is to bring about a change in the pattern of reaction to pain so that, although pain is perceived, it does not bring forth the usual responses, such as anxiety, fear, panic, withdrawal, and flight. For example, in instances of intermittent pain, as during parturition, there is afforded opportunity for relaxation and even sleep between labor pains. With continuous pain, the individual becomes more capable of tolerating the experience when it is freed of its implications.

Consideration of the therapeutically useful quantity and duration of action of the opiates is relevant. It has been shown that the threshold-raising action of the opiates cannot be made to exceed well-defined limits regardless of the quantity administered. It has also been shown that the peak of the threshold-raising effect is reached, regardless of quantity administered, in approximately 90 minutes after administration. To what extent these values are relevant to the aforementioned psychological effects is important. But calibration of these qualities is less precise than threshold-raising action.

Many of the psychological effects pertaining to the pattern of reaction to pain did not have the same time-action curves as did the pain threshold. As described above, therapeutically important psychobiological effects, other than those of threshold-raising action, were attained within 20 minutes of administration and long outlasted the threshold-raising action of the opiates.

It is a common impression derived from bedside experience that the therapeutic effectiveness of a given quantity of opiate can be determined within

30 minutes of the time of administration, and if the patient is by that time still uncomfortable he needs more opiate to achieve a satisfactory therapeutic result. The validity of this position cannot at present be challenged. The question may be raised, however, as to whether the psychobiological effects are maximal at such time, or simply pronounced. A doubt may also be expressed as to whether amounts greater than "saturation" raise therapeutic effectiveness by inducing more satisfactory effects upon the reaction pattern of pain.

Attention may be focussed again upon the fact that pain is a potent antagonist of opiate action. This has been clearly demonstrated as regards threshold raising effect, and less precisely, though definitely, as regards intensity and duration of other psychobiological effects. It is likely, in an analogous way, that pain antagonizes the respiratory depressant action of the opiates. Therefore, if with pain of high intensity larger than the aforementioned quantities of opiate should be used for therapeutic purposes, and if the pain should spontaneously stop the antagonistic effect of pain upon this respiratory depressant action of the opiates will be as suddenly diminished, and serious toxic sequelae may follow.

3 Induction of lethargy and sleep Lethargy and defects in mentation are not the essential components of the action of morphine in relieving anxiety, for with barbiturates extreme lethargy and difficulty in mentation may occur with little diminution in anxiety. Thus, evipal has been observed to induce with lethargy an unpleasant rush of thoughts with reiteration of unresolved personal problems and secondarily induced anxiety not unlike that of delirium. The subject may feel frightened bewildered and uncertain. Furthermore, lethargy and mentation difficulties may accentuate anxiety, possibly because the subject feels inadequate to deal with his problems. However, when combined with freedom from anxiety lethargy by inducing immobility or sleep may be a therapeutic asset. In preliminary experiments, sleep has raised the pain threshold 50 per cent

centimeters of skin surface for 3 seconds. The intensity of radiation, which barely evoked pain was denoted as the pain threshold. The threshold raising action of various opium derivatives was then ascertained in terms of the normal threshold. Morphine sulphate in quantities from 0.1 mgm. to 30 mgm., and codeine phosphate in quantities from 15 mgm. to 240 mgm. were thus assayed.

2 The minimum effective quantity of morphine sulphate was 0.5 mgm. The "saturation" quantity or the smallest amount with which the highest threshold raising effect was attained, was approximately 30 mgm. The "saturation" quantity of codeine phosphate was 60 mgm. The "saturation" level for morphine sulphate, or the highest threshold raising effect of which the drug was capable, was 100 per cent above the control threshold. The "saturation" level for codeine was 50 per cent above the control threshold.

3 The maximum threshold raising effect for quantities of morphine sulphate in 0.5 mgm. to 15 mgm. was reached at approximately the same time that is about 90 minutes after administration. Other opiates tested took approximately the same time.

4 The time-action curve of threshold raising effect for morphine sulphate revealed that essential elimination increased at a constant rate with quantities up to 15 mgm. However with larger quantities of the agent, there was acceleration of the essential elimination rate so that duration of effect with 15 or 30 mgm. of morphine differed but slightly. The time-action curve for codeine phosphate revealed essential elimination at a constant rate up to 60 mgm. With larger amounts there was acceleration of essential elimination.

5 The threshold raising action of dihydromorphinone hydrochloride ('Dilaudid') (3 mgm.), methyl dihydromorphinone ('Metopon') (66 mgm.) and pantopium hydrochloride ('Pantopon') (20 mgm.), was measured. The above amounts of the first two agents had time action curves comparable to that obtained with 30 mgm. of morphine. On this basis, pantopium (20 mgm.) was equivalent to approximately 8 mgm. of morphine sulphate.

6 The threshold raising action of opium derivatives as well as other observable effects, was reduced or obliterated by pain. A uniform pain

SUMMARY AND CONCLUSIONS

1 Quantitative measurements of the pain threshold were made by irradiating 3.5 square

stimulus had a greater neutralizing effect on the threshold-raising action of these agents when the pain occurred just before or during the first 90 minutes after administration of the opiate. Thereafter, pain had less effect on the threshold-raising action. In other words, if pain preceded or occurred early in the course of the action of the opiate, it reduced or neutralized the threshold-raising effect of these agents.

7 This antagonism between pain and the threshold-raising action of opiates was simulated by the administration of a sympathomimetic agent (epinephrine) before the opiate was administered. It is possible that the heightened sympathetic activity, which is secondary to pain, is a factor in the above described antagonism.

8 In normal subjects the outstanding psychological effects of morphine were freedom from anxiety, feelings of contentment and relaxation, apathy, difficulties in mentation, lethargy and sleep. Of especial significance were the emotional states referred to as freedom from anxiety and feelings of contentment. While in these states the subjects perceived pain, but the usual reaction pattern to pain was altered. It is suggested that the presence of an opiate accentuates the ability to dissociate pain perception from the pattern of pain reaction. It is postulated that much of the therapeutic effectiveness of opiates in the management of pain is based on their ability to alter the usual withdrawal fight-flight-anxiety reaction pattern of pain to one of freedom from anxiety, indifference, or apathy.

9 The therapeutic effectiveness of the opiates is dependent mainly on three properties (1) threshold-raising action, (2) the dissociation of pain perception from the usual reaction to pain, and (3) the induction of lethargy and sleep.

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THE AMYLOLYTIC AND PHOSPHATASE ACTIVITY OF LIVER TISSUE IN VON GIERKE'S DISEASE¹

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The pathogenesis of von Gierke's disease is not known Shortly after the disease was described (1), the increased glycogen storage was attributed to a disturbance of the glycogen splitting ferments (2) This stimulated studies of the amylolytic activity of the diseased tissues since amylase was the only glycogen splitting enzyme known at that time Several investigators (3 4 5) found amylase present in the von Gierke tissue Our determinations corroborate these findings as may be seen in Table I

TABLE I
Amylolytic activity of liver tissue

Source of tissue	mgm maltose split by 100 mgm. tissue in 1 hour
von Gierke's disease Case I	2.55
von Gierke's disease Case II	0.70
von Gierke's disease Case III	1.70
Normal liver (Child)	1.75
Normal liver (Adult)	1.45
Fatty liver (Alcoholic cirrhosis)	1.02
Liver from patient with elevated blood amylase activity	3.10

Recently Cori (6) has shown that the conversion of glycogen to glucose in the liver results from the combined action of a phosphorylase which catalyzes the hydrolysis of the glycogen to glucose 1 phosphoric acid ester and a phosphatase which splits the ester to glucose and inorganic phosphate Ostern, Herbert and Holmes (7) report similar findings and demonstrate in addition that the amylase could not account for more than 15 per cent of the glycogenolysis in their experiments In view of this recently demonstrated mechanism for the conversion of glycogen to glucose in the liver and since the amylolytic activity of the liver tissue is probably normal in von Gierke's disease, the possibility of a disturbance in the phosphorylase-phosphatase system was considered

Liver tissue from three fatal cases of von Gierke's disease was examined for its phosphatase and amylolytic activity For comparison parallel experiments were carried out with tissue obtained from normal livers and from livers showing marked fatty change The latter were chosen for comparative studies because the abnormally high fat content of the von Gierke liver made it necessary to consider the possibility of the fat interfering with the normal phosphorylase-phosphatase conversion of glycogen The fatty livers contained 38.5, 25, and 34 per cent fat (determined as fatty acids in dried tissue) The fat content of the von Gierke livers was 52.34, 34.2, and 22.6 per cent, the corresponding glycogen content was 27, 12.7, and 36.6 per cent (of the dried tissue weight) Total wet weights of these latter livers were 1216 grams, 1063 grams, and 250 grams respectively Detailed data concerning the partition of the liver tissue lipid content will be published subsequently

The enzyme preparations consisted of dried powdered liver obtained by freezing (8) The final product was washed with small portions of acetone and dried again in a desiccator The alkaline phosphatase activity was determined by a modification of the Bodansky method (9), using 20 milligrams of dried liver powder and a 24-hour period for hydrolysis The method was also employed in determining the acid phosphatase activity except that an N acetate buffer (pH 5.0) was substituted for the alkaline buffer The phosphatase values obtained are shown in Table II

Aqueous extracts of the powdered tissue were used in determining the amylolytic activity The latter was measured with a method described elsewhere (10) Since the method utilizes starch as a substrate and a phosphate buffer it may not measure the activity of the amylase alone. Even though adenylic acid considered to be essential for the phosphorylation reaction (Cori), was not added the reducing substances measured may have included glucose. However, amylolytic ac-

¹ Work aided by a grant from the Rockefeller Foundation the Bingham Associates Fund, and the Charlton Fund.

² Emanuel Libman Fellow

TABLE II
Phosphatase activity of liver tissue*

Source of tissue	Alkaline phosphatase	Acid phosphatase
von Gierke's disease, Case I	0 117	1 176
von Gierke's disease, Case II	0 063	0 500
von Gierke's disease, Case III	0 250	1 538
Normal liver (Child A)	0 563	1 050
Normal liver (Child B)	0 531	1 163
Normal liver (Adult A)	0 425	2 050
Normal liver (Adult B)	0 353	1 310
Fatty liver (Sub acute bacterial endocarditis) (Child)	0 350	2 300
Fatty liver (Alcoholic cirrhosis)	0 279	1 050
Fatty liver (Infant) (Increased glycogen not of von Gierke origin)	0 300	2 600
Patient with elevated blood phosphatase (alkaline)	1 013	0 750

* The values represent milligrams of phosphorus split by 20 milligrams of powdered tissue in 24 hours

tivity was demonstrated by qualitative iodine tests during the course of the amylase hydrolysis. Color changes indicative of the formation of dextrans were obtained.

As may be seen in Table II, the alkaline phosphatase activity of the von Gierke liver tissue was definitely decreased as compared to that of the normal livers of children. The range of alkaline phosphatase activity in fatty livers of both children and adults was somewhat lower than that in the normal liver tissue of corresponding groups. In tissue from a fatty liver which contained a relatively large amount of glycogen (8 per cent), but which was not from a genuine case of von Gierke's disease, the alkaline phosphatase activity was also decreased as compared to that in the normal liver tissue of children.

The acid phosphatase activity in each liver except one (liver from a patient who had markedly elevated alkaline blood phosphatase activity) was greater than the alkaline phosphatase activity. However, no definite ratio was found, nor was any alteration of the acid phosphatase noted in von Gierke's disease.

The identity of the glucophosphatase with the alkaline glycerophosphatase has not been definitely established. Hexosephosphate is hydrolyzed by bone phosphatase (11). Liver phosphatase splits glycerophosphate, glucose-1 and glucose-6 phosphate at about the same rate (6). It is still uncertain if, in addition, a phosphatase exists that specifically dephosphorylates the Cori ester (7). Decisive information concerning the nature of the phosphatases is still lacking.

A disturbance of the phosphatase which would leave the glucose-phosphates unsplit and therefore available for glycogen resynthesis could result in an increased storage of the polysaccharide. A decrease of the phosphatase activity by lowering the amount of free phosphate would also favor glycogen storage by retarding glycogenolysis. We wish to call attention to the lowered alkaline phosphatase activity of the liver tissue in three cases of von Gierke's disease as compared to that of normal livers of two children. A subsequent report will deal with glycogenetic and phosphorylase-phosphatase glycogenolytic studies of tissue in von Gierke's disease, which are now in progress.

Values for total wet weight, total glycogen, total fat, and total nitrogen are not available in this series but may be studied in the future in order that the enzyme activity may be referred to the total parenchymal liver tissue.

SUMMARY

A report is made of the amylase and phosphatase activity of liver tissue in von Gierke's disease with a comparison of the activity of these enzymes in normal liver tissue and tissue of fatty livers.

The presence of amylolytic activity in von Gierke liver tissue is corroborated.

The alkaline phosphatase activity of the liver tissue in three cases of glycogen storage disease was found to be significantly lower than that present in normal livers of two children.

The authors wish to express their thanks to Dr Kenneth D. Blackfan and Dr Sidney Farber of the Children's Hospital in Boston and to Dr Elmer Barron and Dr Francis MacDonald of the Boston Floating Hospital for their cooperation in making the organs of the von Gierke patients available for our studies. Much of the other material was obtained through the kindness of Dr Timothy Leary.

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THE MECHANISM OF THE EXCRETION OF VITAMIN C BY THE HUMAN KIDNEY AT LOW AND NORMAL PLASMA LEVELS OF ASCORBIC ACID¹

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(Received for publication December 13 1940)

In a previous study (1) the excretion and renal tubular reabsorption of vitamin C at normal and artificially elevated plasma concentrations of the vitamin were reported.

Refinements in the chemical determination of vitamin C in plasma and urine (2, 3) have made it possible to further supplement our previous data with observations at the lower ranges of the plasma concentration. On the basis of the previous evidence it was concluded that the excretion of vitamin C at any plasma concentration was determined by the plasma concentration by the rate of glomerular filtration and by the rate of tubular reabsorption. It was further observed that the reabsorptive mechanism was limited by a maximal rate and that, when the vitamin was presented to the tubules by the glomerular filtrate at a rate exceeding this maximum, the excess was excreted in the urine. The observations we are reporting in this study are concerned with the nature of the reabsorptive process at the lower plasma levels of vitamin C. In the previous studies the plasma concentrations of the vitamin varied from 1.5 to 25 mgm. per cent. In the present study the plasma concentrations varied from 0.03 to 2.05 mgm. per cent.

PROCEDURE

Nineteen normal individuals were used in this study. Clearances were determined at constant plasma levels of the vitamin, and only one level was studied on any day. When necessary, vitamin C was given orally the night prior to and the morning of the experiment in order to obtain the desired plasma concentration. Fluids were forced on the day prior to the test, and on the morning of the experiment the subject drank 1000 cc. of normal saline. The first clearance period started 1½ to 2 hours after the last dose of water. The bladder was catheterized and washed out with saline. This specimen of urine was discarded. The catheter was left *in situ* and

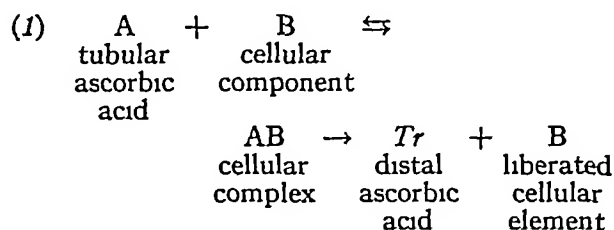
clamped. At 30 or 60-minute intervals urine specimens were collected and the bladder washed out with saline and air. In a previous study it was found (16) that no destruction of ascorbic acid occurred in bladder urine for periods as long as 5 hours. It seemed therefore perfectly safe to allow the urine to collect in the bladder for 30- to 60-minute intervals. Bloods were taken at frequent intervals during the clearance periods. The urines as collected were immediately acidified with glacial acetic acid and kept in the refrigerator until analyzed. No longer than 2 hours elapsed before analysis. Vitamin C in urine was determined in the photoelectric colorimeter by the method described by Evelyn *et al* (2). With the titrimetric method it was difficult to determine the vitamin C clearance at low plasma concentrations due to the interference of relatively large amounts of non-vitamin C reducing substances in the urine. Since vitamin C is the only known substance which will react with dichlorophenolindophenol immediately the Evelyn technique provides a satisfactory method for distinguishing between vitamin C and any non-vitamin C-reducing substances. Other substances reacting with the dye do so at a fairly slow rate. Where very small amounts of non-vitamin C reducing substances are present, or where large concentrations of ascorbic acid necessitate dilutions, then the error in the determination of urinary ascorbic acid is of the magnitude of ± 2 per cent. Where the concentration of reducing substances other than vitamin C is fairly large, or where there are very small amounts of ascorbic acid present, then the percentage error is increased. In pure solutions of ascorbic acid, when the concentration ranges up to 2.00 mgm. per cent, duplicate analyses agree within 0.03 mgm. per cent, so that, if the absolute concentration is 2.00 mgm. per cent, there is a 1.5 per cent error if 1.00 mgm. per cent, a 3 per cent error and if 0.50 mgm. per cent, a 6 per cent error. When non-vitamin C reducing substances are present, the method of extrapolation required increases this error.

Plasma vitamin C was determined by the method of Mundlin and Butler (3). We have found it possible to consistently reproduce plasma figures within 0.04 mgm. per cent. The absolute magnitude of the error is constant over the entire range of plasma values.

Simultaneous inulin clearances were not done in this study because the magnitude of the vitamin C clearances at these low plasma levels was such that the variations in the normal inulin clearance could not appreciably affect the vitamin C/inulin clearance ratio. In calculating the

¹ This research was aided by a grant from the Josiah Macy Jr. Foundation.

follow from the circumstance that, in the process of active transfer, the substance enters into reversible combination with some cellular element present in a limited and constant amount, and it is the rate of decomposition of this complex which limits the progress of the overall reaction of transfer. In the case of vitamin C this situation may be represented as follows



Applying the law of mass action to such a situation, one may derive the following relationship (15)

$$(2) \quad K = (a - Tr/V) \left(\frac{Tm - Tr}{Tr} \right),$$

where Tm represents the maximal rate of tubular reabsorption, V the rate of glomerular filtration in units of 100 cc, a the plasma concentration, Tr the calculated rate of tubular reabsorption, and K a constant

In our previous work we attempted to describe the excretory process according to this hypothesis. In the application of equation 2, when V , the rate of glomerular filtration, was reduced to 100 cc per minute, the value for Tm used was 1.77 mgm per 100 cc of glomerular filtrate. This figure for Tm was the average value obtained for the 4 individuals in the previous study. However, Tm has been found to vary in normal individuals and even in the same individual at different times. For example, Smith and his co-workers (6) and we in our studies have observed Tm values ranging from 1.20 mgm to 2.10 mgm of vitamin C per 100 cc of glomerular filtrate. This variation in Tm is not only true for vitamin C but also for glucose, as observed by Shannon (7).

In Figure 3 the data from the previous study

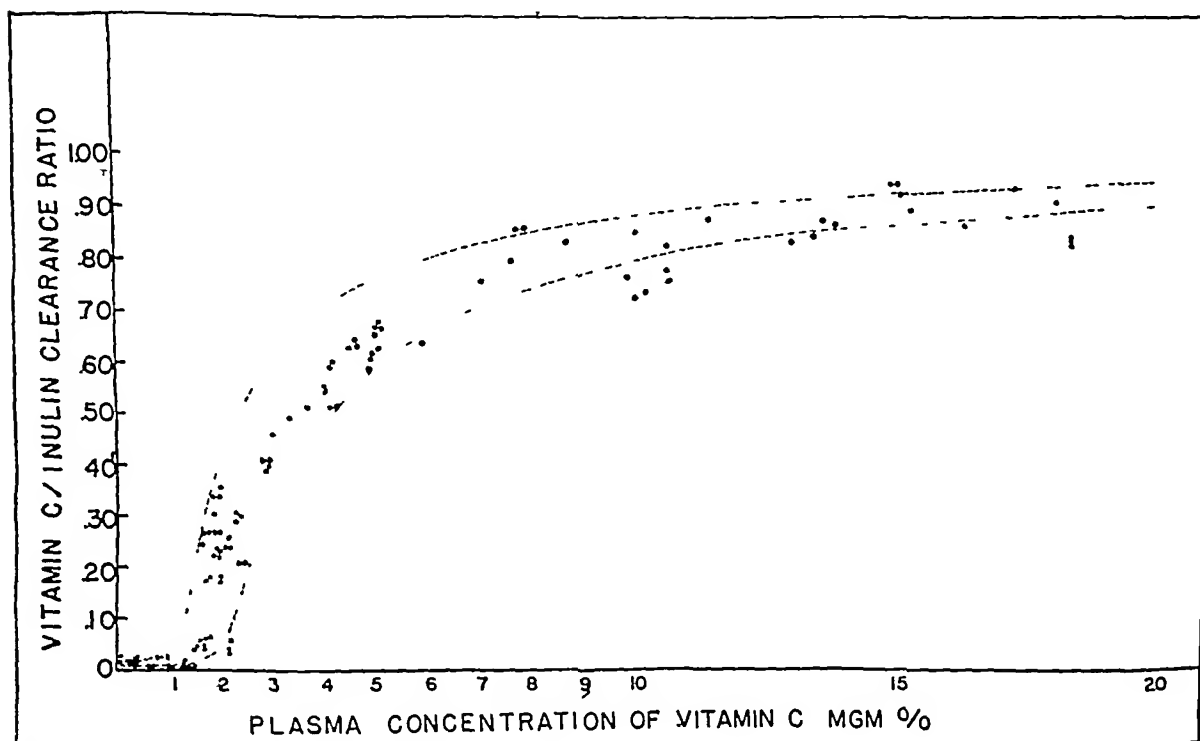


FIG. 3 VITAMIN C/INULIN CLEARANCE RATIOS PLOTTED AGAINST PLASMA CONCENTRATION OF VITAMIN C

The two lines are calculated from equation 2 given in the text. The dots represent the actual individual clearance ratios

(1), which included the higher plasma concentrations of vitamin C, are plotted along with the present observations. The two smooth curves were calculated using the following equation

$$(3) \text{ vitamin C/inulin clearance ratio} = 1 - Tr/a$$

The values for Tr were obtained by using equation 2 in which the values for T_m were 1.20 mgm./100 cc. and 2.10 mgm./100 cc. of glomerular filtrate, V was equal to 100 cc per minute and K had a value of 0.01. This value for K was taken in preference to the one of 0.1 used in the previous study because it defined the reaction throughout the entire variation in Tr .

The data obtained at low, normal and high plasma concentrations of vitamin C fit this calculated zone (Figure 3) satisfactorily and indicate that the hypothesis upon which equations 1 and 2 are based can be used to describe the reabsorptive system at all plasma concentrations examined. The data further suggest (1) that the vitamin C clearance does not become zero at low plasma levels and, if equation 2 does adequately describe the system, there exists a minimal value, which is independent of the plasma level and which is of very low magnitude (2) that as the plasma concentration rises and approaches the value for T_m the excretion of vitamin C rises rapidly, and (3) that at very high plasma concentrations the vitamin C clearance approaches the inulin clearance.

SUMMARY

Sixty nine observations of the clearance of vitamin C at plasma levels varying from 0.03 to 2.05 mgm. per cent suggest that the reabsorption of the vitamin is never complete and that the clearance of vitamin C at the low plasma levels is constant and independent of the plasma concentration.

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A PHOTOELECTRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF ERYTHROCYTE FRAGILITY

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(Received for publication April 27 1940)

The method described in most textbooks for the determination of the fragility range of erythrocytes possesses several disadvantages. In the first place, quantitation is not possible because unmeasured drops of blood are placed in tubes containing only roughly estimated amounts of hypotonic salt solution. As might be expected, a semi-quantitative technique of this type, when applied to clinical investigation, gives grossly irregular fragility curves such as those obtained by Vaughan (1). Secondly, since the observed degree of hemolysis in any one tube is dependent upon diffusion of liberated oxyhemoglobin through the liquid above the sedimented cells, other factors such as temperature, the anticoagulant used, the amount of bile pigments present in the plasma, and the presence of hemolytic organisms may affect the results erroneously. It seemed desirable, therefore, to devise for this test a technique rapid and simple enough for clinical application, and at the same time sufficiently accurate for purposes of research.

Because of the fact that the amount of oxyhemoglobin liberated by a hypotonic salt solution is a direct function of the number of cells hemolyzed, it is obvious that a determination of the freed oxyhemoglobin resulting from such partial hemolysis may be used as a hemolytic index. Furthermore, as complete hemolysis results from laking erythrocytes in a 0.10 per cent solution of sodium carbonate (2), it is possible to express the hemolysis occurring in a given strength of salt solution in terms of total hemolysis. Thus, the quantitative determination of partial hemolysis in any strength of salt solution can be easily made if a measured amount of blood is placed in a centrifuge tube containing a definite quantity of the salt solution and, after partial hemolysis has been completed, the unhemolyzed cells are centrifuged off and the oxyhemoglobin in solution in the supernatant fluid estimated by means of a photoelectric colorimeter. Since the technique of the latter instrument is well established (2), it need not be outlined here.

METHOD

Place 1 cc. of a filtered 1.6 per cent solution of sodium oxalate in a test tube. Boil over an open flame until all water and vapor have been driven off. With a clean dry syringe and needle obtain exactly 5 cc. of blood from an arm vein and transfer quickly to the prepared test tube. Mix at once to prevent clotting.

For the determination of partial hemolysis, prepare twenty six stock solutions of sodium chloride decreasing its strength by 0.02 per cent from 0.70 per cent to 0.20 per cent, inclusive. These must be accurately made and must be kept in well stoppered bottles. Into one of twenty six¹ clean centrifuge tubes transfer exactly 10 cc. of the 0.70 per cent salt solution and label and in a like manner place 10 cc. of the other salt solutions in the remaining tubes. With a volumetric pipette transfer exactly 0.05 cc. of the well mixed oxalated blood to each tube, place a rubber cap on each, and invert several times to obtain complete mixing. Allow to stand at room temperature for at least thirty minutes then invert each tube again, place in a centrifuge, and spin at moderate speed (1500 r.p.m.) for fifteen minutes. Remove the tubes from the centrifuge, carefully decant the supernatant fluid from each, and transfer separately to clean photoelectric absorption cells. Using an appropriate color filter make determinations of the light transmission, and read off the grams of oxyhemoglobin from a previously prepared photoelectric conversion graph.

To obtain complete hemolysis place 10 cc. of a 0.1 per cent solution of sodium carbonate in a test tube and transfer to it exactly 0.05 cc. of the same blood and mix immediately. Fill a photoelectric absorption cell with this solution, and in a similar manner determine the grams of oxyhemoglobin present. If the latter figure is low more accurate results may be obtained by using 0.1 cc. of blood both in the sodium carbonate and in the hypotonic salt solution tubes.

Calculation. Divide the number of grams of oxyhemoglobin resulting from hemolysis in each centrifuge tube by the number of grams obtained from complete laking with sodium carbonate. These figures represent the per cent of total hemolysis occurring in each strength of salt solution.

Plotting of curves. On rectangular coordinate paper mark off the salt solutions in order of decreasing strength along the abscissa, and the per cent hemolysis in increasing order along the ordinate. Plot the per cent hemolysis

¹ In respect to a particular blood, if some idea is obtained beforehand of where hemolysis is apt to begin, the number of solutions may be reduced in number.

sis for each salt solution and connect the points in a continuous curve. If the technique has been carefully carried out, the curve will ordinarily be sigmoid in character—that is, convex toward the base at lower percentages, and convex upward as hemolysis nears completion. In some instances, particularly in the case of bloods showing increased fragility, the curve may show more than one maximal slope. The significance of this occasional variation from the usual curve, however, is by no means clear and requires further investigation.

DISCUSSION

Sigmoid curves such as these represent the first integral of curves of the probability type, or, in other words, the plotted first differential of such sigmoid curves gives the probability-distribution of the erythrocyte fragility of any particular blood for varying strengths of salt solution. As has been shown by Ponder (3), when the per cent of total hemolysis is plotted against time, various hemolytic agents give typical sigmoid curves. Moreover, if one plots Ponder's values for the per cent hemolysis produced by varying concentrations of a lysin at a given time interval after lysis has begun, it can be demonstrated that each of the resulting curves is also sigmoid in character. One can affirm, therefore, that hypotonic solutions behave in this respect like other lysins.

The curve of normal hemolysis in Figure 1 was derived from fragility determinations of the erythrocytes of twelve normal individuals. The per cent hemolysis for each strength of salt solu-

tion was first averaged for the twelve bloods, and these averages were then plotted on arithmetic probability paper (Figure 2) (4). In general, the points fell on a straight diagonal line, and those not so doing exhibited only small deviations to either side. Thus, after taking from the probability coordinate corrected points for the per cent hemolysis for the salt concentrations in question, the *normal* curve was constructed on rectangular coordinates as shown.

It will be seen that normal blood shows less than 2 per cent hemolysis in 0.50 per cent salt solution and reaches a maximal hemolysis at 0.34 per cent. If every blood showed an equal deviation from the mean fragility, all curves, of course, would be parallel to each other regardless of their position in respect to the abscissa. That this does not occur is obvious, and hence it is impossible to construct a whole curve from the determination of the per cent hemolysis produced by a single concentration of salt. Nevertheless, by using only three appropriate strengths of salt (*viz.* 0.52, 0.42, 0.32 per cent), the position of any curve can be approximated with minimal effort, and for most clinical purposes this should suffice.

In Figure 1 the curve beginning at high salt concentrations represents a case of hemolytic jaundice, while the two lying to the right of the normal curve exemplify the fragility range of a case of Cooley's anemia and of a case of chronic benzol poisoning. Curves have also been ob-

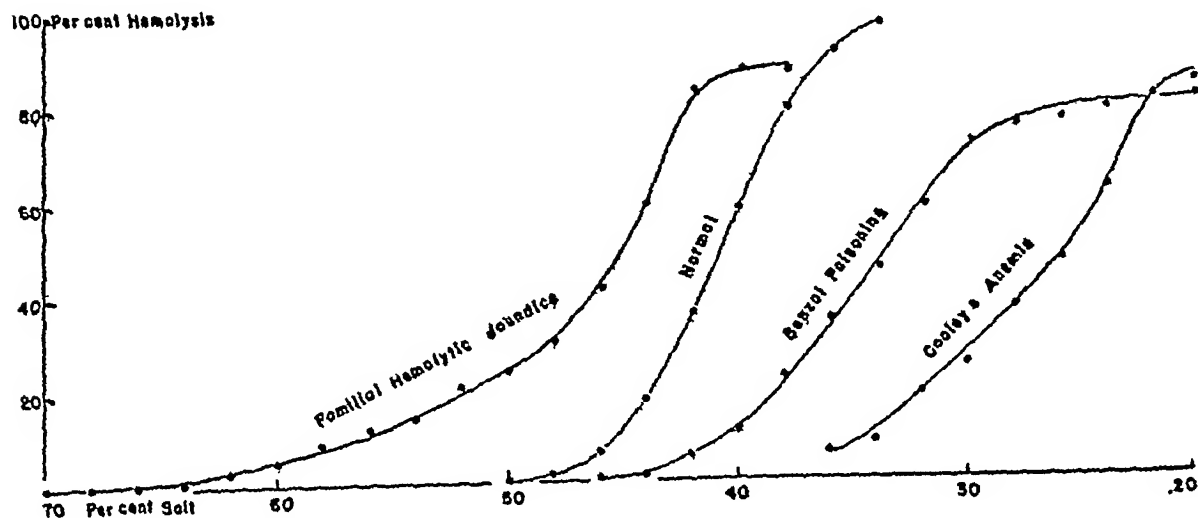


FIG 1 PER CENT HEMOLYSIS PLOTTED AGAINST STRENGTH OF SALT SOLUTION

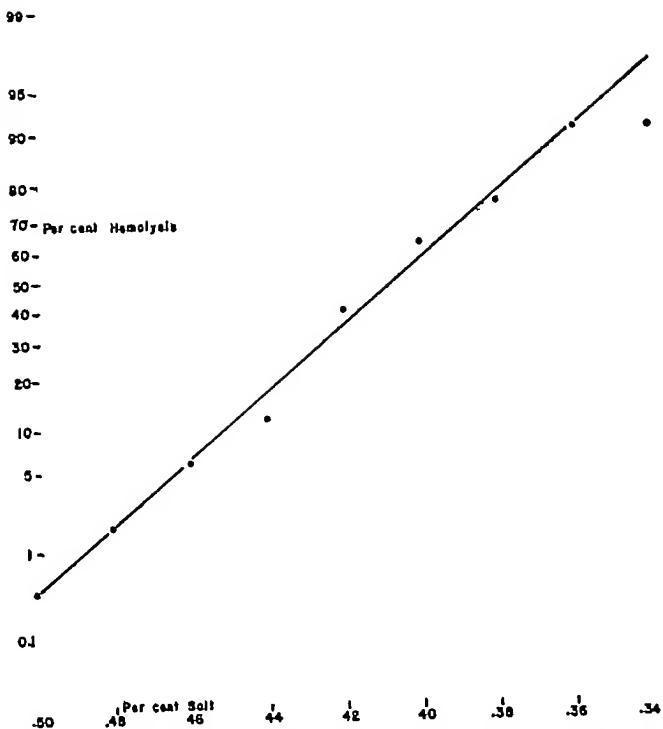


FIG. 2. PER CENT HEMOLYSIS PLOTTED ON PROBABILITY PAPER AGAINST STRENGTH OF SALT SOLUTION

These points represent the average hemolysis in 12 normal individuals for varying salt strengths

tained from additional cases of hemolytic jaundice and from various other disorders of the hematopoietic organs but, since these showed the same general pattern it did not seem worth while to reproduce them here.

For clinical purposes it has been found that increased fragility can be easily ruled out by placing 0.05 cc. of blood in 10 cc. of 0.50 or 0.52 per cent salt solution and centrifuging. If the supernatant fluid shows no evidence of hemolysis to the naked eye, the fragility is normal or less than normal. It has also been observed that, due to the high dilution of blood (1:200) here employed when the total oxyhemoglobin of the

whole blood is near normal the presence of an icteric index even as high as 100 raises the oxyhemoglobin values by only 4 or 5 per cent. But when the oxyhemoglobin content of the blood is markedly decreased, the presence of any considerable degree of jaundice introduces an error sufficient at times to warrant replacing the plasma with 0.85 per cent salt solution before the test for fragility is undertaken.

SUMMARY

1. A photoelectric method for erythrocyte fragility is described.

2 The sigmoid curves obtained by this technique represent the first integral of the probability-distribution of fragility in respect to concentration of salt

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ON THE INACTIVATION OF THROMBIN BY PLASMA PROTEIN

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(Received for publication May 1, 1940)

It has been known for many years that an excess of thrombin is formed during the clotting of blood or plasma, that the excess is rapidly inactivated, and that such inactivation is important in maintaining normal fluidity of the blood (1). Considerable attention has been given to the question of what happens to the excess of thrombin, but little conclusive information has been obtained concerning the phenomenon. Quick, in a study of the mode of action of heparin in preventing coagulation obtained evidence supporting the opinion of early workers that the property of inactivating thrombin resides in the plasma protein,

and specifically in the albumin fraction (2). The present report deals with a study of the rate of inactivation of thrombin *in vitro* by plasma protein and various fractions of plasma protein.

METHODS

The human and beef plasma samples used in this study were prepared by adding one part of 1.85 per cent $K_2C_2O_4$ solution to seven parts of fresh blood and centrifuging immediately. A potent thrombin solution was prepared from beef plasma by the method of Eagle (3). The thrombin solution was ampuled and preserved in a cold box at $-40^\circ C$. and, except for the experiments of Figures 2 and 6, the same lot was used throughout.

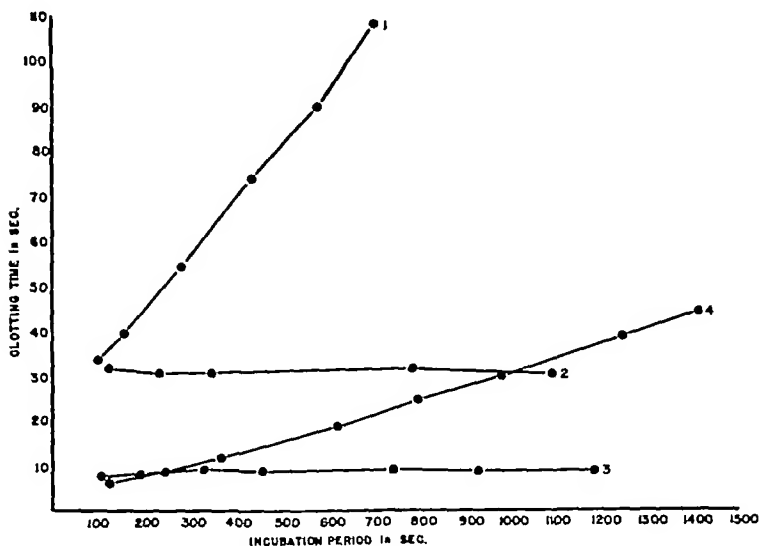


FIG. 1. THROMBIN INACTIVATION STUDY Ox PLASMA AT $37^\circ C$.

Curve 1. 2.0 cc. thrombin solution plus 0.2 cc. ox plasma incubated, 0.2 cc. mixture added to 0.4 cc. fibrinogen solution. Curve 2. 2.0 cc. thrombin solution plus 0.2 cc. fibrinogen solution, 0.2 cc. mixture added to 0.4 cc. fibrinogen solution. Curve 3. 2.0 cc. thrombin solution plus 0.2 cc. fibrinogen solution, 0.2 cc. mixture added to 0.4 cc. ox plasma. Curve 4. 2.0 cc. thrombin solution plus 0.2 cc. ox plasma, 0.2 cc. mixture added to 0.4 cc. ox plasma.

the study No loss of strength was noted, and 0.1 cc. of the thrombin solution clotted 0.5 cc. of human plasma in 10 seconds. The total nitrogen content of the thrombin solution was 77 mgm per cent. Fibrinogen solution was prepared from beef plasma by adding one-third volume of saturated $(\text{NH}_4)_2\text{SO}_4$, centrifuging, dissolving the precipitate in 0.9 per cent NaCl solution, and subsequent reprecipitation twice. The $(\text{NH}_4)_2\text{SO}_4$ was removed by dialysis against 0.9 per cent NaCl solution, and the final volume of the fibrinogen solution was about one-third that of the original plasma. The horse serum and fractions of horse serum protein studied will be described below.

The measurement of the rate of inactivation of thrombin by the protein solution under study was made in every experiment as follows. Two cc. of thrombin solution were placed in a serological test tube in a water bath at 37°C and 0.2 cc. of the solution being tested was added. The clot which formed when plasma or fibrinogen solution was added was rolled out and it was found to make no difference whether the fibrin pellicle was left in the incubating mixture or not. At various intervals 0.2 cc. of the incubating mixture was withdrawn and combined with 0.4 cc. fresh human or beef plasma or fibrinogen solution, and the clotting time was measured with a stopwatch. The determination thus consisted of the measurement of the residual thrombin by noting its power to clot

a standard substrate. As previously shown (4), a strict linear relationship between thrombin concentration and clotting time under these circumstances does not exist, but for the purposes of the present study this assumption may be made with little error.

PRESENTATION OF DATA

In the experiments on which Figure 1 is based, the inability of fibrinogen solution to inactivate thrombin is brought out. In curves 2 and 3 fibrinogen solution was added to thrombin and immediate clotting resulted. Nevertheless no inactivation followed. In curves 1 and 4, by contrast, the inactivating power of plasma is well shown. In the test for residual thrombin the fibrinogen solution clots uniformly more slowly than does fresh plasma.

Figure 2 shows composite inactivation curves obtained in a study of human plasma from two individuals over a period of several weeks. In Figure 3 is shown a composite of 16 inactivation curves made by studying plasma samples from the same individual over a period of several weeks.

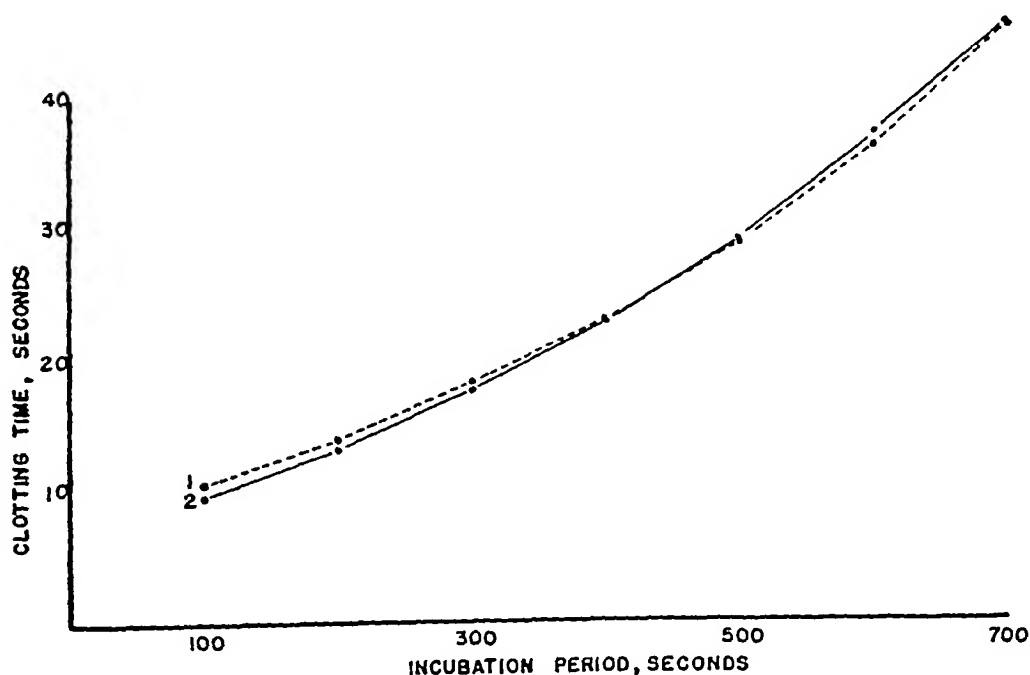


FIG 2 THROMBIN INACTIVATION STUDY, AT 37°C , COMPOSITE CURVES

1 J.D.S., control, 12 curves 2 G.M.R., control, 12 curves.

Curve 1 2.0 cc. thrombin solution plus 0.2 cc. fresh human plasma incubated, 0.2 cc. mixture added to 0.4 cc. fresh human plasma. Composite of 12 such curves. Curve 2 Similarly made from second control.

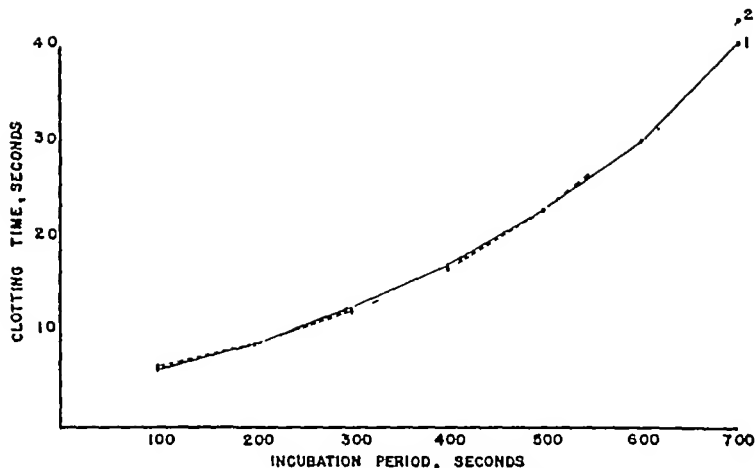


FIG. 3 THROMBIN INACTIVATION STUDY AT 37° C.

1 J D.S., control, composite 16 curves. 2. Theoretical curve $y = (4.59) (1.003)^x$

Curve 1 2.0 cc. thrombin solution plus 0.2 cc. fresh human plasma, incubated, 0.2 cc. mixture added to 0.4 cc. fresh human plasma. Composite of 16 such curves. Curve 2. Derived theoretical curve.

Shown also as curve 2 is the theoretical exponential curve derived from two values on the composite curve. In Figure 4 is shown the effect of dialysis and heat on the thrombin inactivating power of human plasma. The plasma tested in curve 2 was dialyzed for 3 hours at 4° C against repeated changes of 0.9 per cent NaCl solution. The slight reduction in inactivating power compared with the undialyzed plasma may be explained by the small increase in volume during dialysis for which no correction was made. Plasma heated to 60° C for 20 minutes under-

went great reduction in inactivating power, while plasma heated to 66° C for 20 minutes completely lost this property

Figure 5 and Table I show data obtained in a study of thrombin inactivating power of horse serum and protein fractions of horse serum both of which were kindly supplied by Drs E J Cohn and T L McMeekin of the Department of Physical Chemistry at Harvard Medical School. The protein fractions were prepared by precipitation from horse serum with varying concentrations of $(\text{NH}_4)_2\text{SO}_4$, as shown in Table I. After filtration the precipitates were taken up in 0.9 per cent NaCl solution and freed of $(\text{NH}_4)_2\text{SO}_4$ by dialysis. The final protein concentrations of the fractions tested are shown in grams per cc. and the values are similar to those of serum. Protein fractions 1 and 2 have no inactivating power, while fractions 3 and 4 exhibit the property to an increasing degree. Fraction 5 precipitated at 2.80 M $(\text{NH}_4)_2\text{SO}_4$ is by far the most active fraction tested against thrombin. Fraction 6, however, soluble at 2.80 M $(\text{NH}_4)_2\text{SO}_4$ and precipitated at saturation has no inactivating power. The meas-

TABLE I

Horse serum protein fractions used in experiments shown in Figure 5. Dissolved in 0.9 per cent NaCl solution

Curve	Protein fraction $(\text{NH}_4)_2\text{SO}_4$ M	Protein concentrations gram per cc.
1	1.370	0.0765
2	1.638	0.0793
3	2.080	0.0891
4	2.60	0.0815
5	2.80	0.0613
6	Soluble at 2.80	0.0570

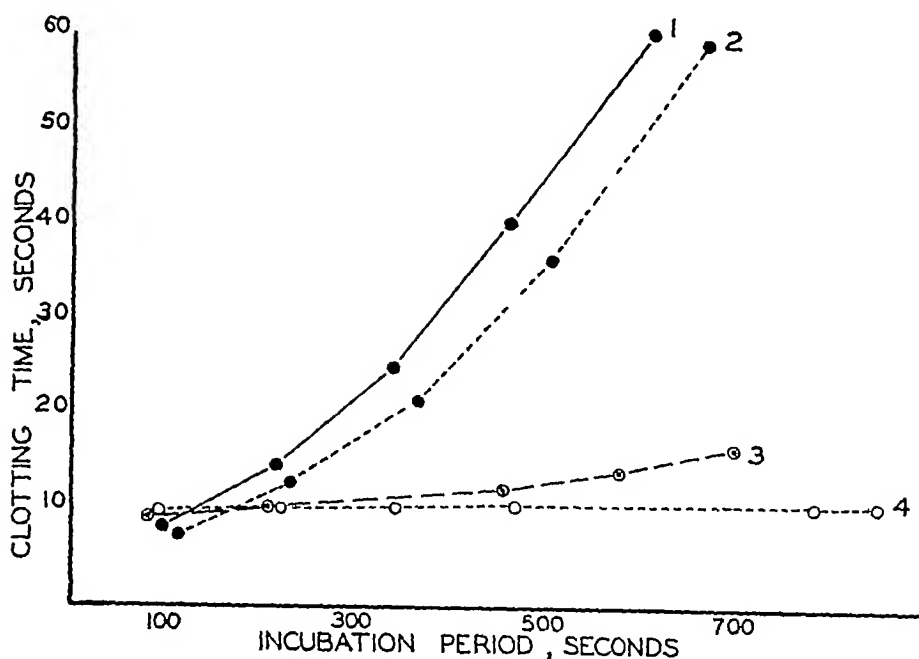


FIG 4 THROMBIN INACTIVATION STUDY, HUMAN PLASMA, 37° C

1 Normal 2 Dialyzed. 3 Heated 20 minutes at 60° C 4 Heated 20 minutes at 66° C

Curve 1 20 cc. thrombin solution plus 0.2 cc fresh human plasma, incubated, 0.2 cc. mixture added to 0.4 cc. fresh human plasma. Curve 2 Same experiment after dialysis of plasma against 0.9 per cent NaCl solution, using undialyzed plasma in test for residual thrombin. Curves 3 and 4 Plasma heated, filtered and tested as in Curve 1 using unheated plasma in test for residual thrombin.

urement of residual thrombin in these experiments was made with fresh human plasma.

The experiments of Figure 6 indicate that the solution of the horse serum protein fraction soluble at 2.00 M $(\text{NH}_4)_2\text{SO}_4$ and precipitated at saturation possesses greater power to inactivate thrombin than does serum itself. The protein fraction was dissolved in 0.9 per cent NaCl solution and, since the protein content was 0.0694 gram per cc as compared with 0.0650 gram per cc for the serum itself, the inactivating property has obviously undergone concentration. The protein precipitated at 2.00 M $(\text{NH}_4)_2\text{SO}_4$ and taken up in 0.9 per cent NaCl solution to make 0.0786 gram of protein per cc has little antithrombic power. The fractionation of serum protein for these experiments was done at 4° C, using the technique of T. L. McMeekin (5).

In other experiments it was found that a hemocypren solution containing 0.0220 gram of protein per cc showed no inactivating power against the standard thrombin solution when tested as de-

scribed above. A solution of horse serum crystallin also was found to be without inactivating power. The question of the possible importance of inorganic constituents of serum in the inactivation phenomenon was also studied. Horse serum was ashed with sulfuric acid, the residue was taken up in a volume of distilled water equal to that of the original serum, and the pH was adjusted to 7.4. The resultant clear solution had no power to inactivate thrombin, from which one may conclude that the antithrombic activity of serum does not reside in its inorganic components.

In what manner vitamin K-active substances, such as 2-methyl-1,4-naphthoquinone, condition the formation of prothrombin in the body has not yet been determined, but it is possible that such substances supply a prosthetic group for the globulin molecule. The possible relationship of vitamin K to the thrombin-inactivation phenomenon under study was investigated as follows. Two cc of the standard thrombin solution were inactivated by incubation with 0.2 cc horse serum for

10 minutes at 37° C. Then 0.4 mgm of a highly K active water-soluble sodium bisulfite derivative of 2-methyl-1,4-naphthoquinone (probably the sodium salt of the 3-sulfonic acid addition product) was added. No restoration of thrombin potency occurred on incubating the solution at 37° C. Addition of the vitamin K substance to the thrombin solution before the serum was added did not affect the rate of inactivation of the thrombin

DISCUSSION

The lack of species specificity in the interaction of the different mammalian clotting factors studied in these experiments is clear. There may be quantitative differences in horse, beef, and human plasma with respect to these factors but the present study is confirmatory of the usual view that the reaction between thrombin and fibrinogen from various mammalian sources is essentially the same. It is apparent also that the inactivating

power of plasma protein is exerted against thrombin from plasma of another species.

Evidently the inactivation of thrombin does not depend on conditions set up by the conversion of fibrinogen to fibrin, for the precipitation of purified fibrinogen by thrombin is not followed by progressive reduction in potency of the residual thrombin. Rapid inactivation results if preserved horse serum is added to thrombin, as shown in the present study. It is not surprising then, to find that immediate removal of the clot after addition of plasma to thrombin has no effect on the rate of inactivation of thrombin.

As shown above the inactivation of thrombin by plasma proceeds exponentially. In studying plasma from the same normal human subjects repeatedly over a period of time, the inactivation rate is remarkably constant. The agreement between plasma samples from different normal individuals is close. These findings led to a study of the inactivating property in plasma from patients

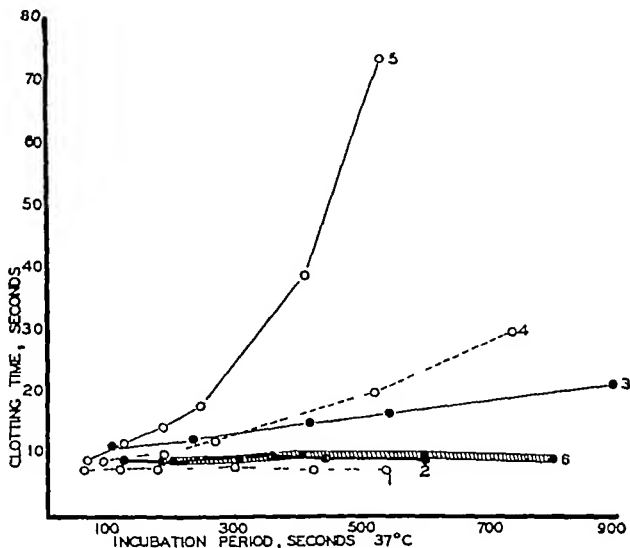


FIG. 5 THROMBIN INACTIVATION STUDY, HORSE SERUM PROTEIN FRACTIONS

Curve 1. 2.0 cc. thrombin solution plus 0.2 cc. solution of protein fraction, incubated, 0.2 cc. mixture added to 0.4 cc. fresh human plasma. Curves 2, 3, 4, 5 and 6 similarly obtained with corresponding protein fractions, as shown in Table I.

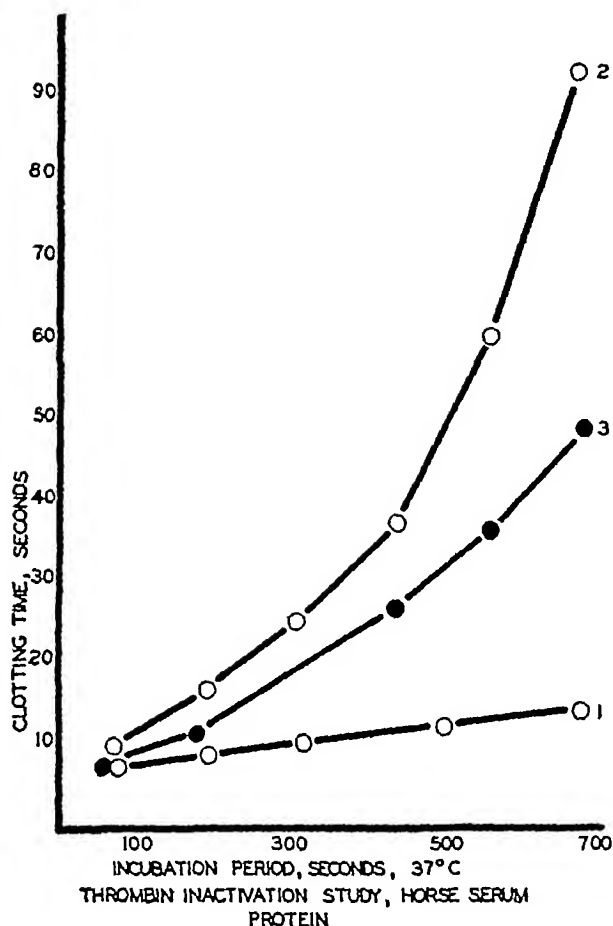


FIG 6. THROMBIN INACTIVATION STUDY, HORSE SERUM PROTEIN

Curve 1 20 cc. thrombin solution plus 0.2 cc. solution horse serum protein fraction (fraction precipitated at 20 M. $(\text{NH}_4)_2\text{SO}_4$, and dissolved in 0.9 per cent NaCl solution to yield 0.0786 gram of protein per cc.) Mixture incubated, and 0.2 cc. mixture added to 0.4 cc. fresh human plasma. Curves 2 and 3 similarly constructed, Curve 2 with solution of horse serum protein fraction soluble at 20 M $(\text{NH}_4)_2\text{SO}_4$ but precipitated at saturation and containing 0.0694 gram of protein per cc in 0.9 per cent NaCl solution, Curve 3 with untreated horse serum containing 0.0650 gram of protein per cc.

with various diseases, the results of which are to be reported

From the data shown in Figure 4 it can be seen that the power of inactivating thrombin is not lost when plasma is dialyzed against 0.9 per cent NaCl solution. This fact, together with the demonstrable heat lability of this property in plasma, makes it clear that thrombin inactivation is a function of the plasma proteins. The studies

on serum protein fractions shown in Figures 5 and 6 indicate a localization of the antithrombic property not merely in the albumin fraction, but in a particular part of the albumin fraction. By far the most active fraction studied was that precipitated at 2.80 M $(\text{NH}_4)_2\text{SO}_4$.

It is of interest that a fraction of horse serum protein can be salted out which yields a solution of greater antithrombic power than the serum itself. It is possible that the power to inactivate thrombin is the property of an individual protein constituent of the plasma albumin, and that the reaction is enzymatic in nature.

CONCLUSIONS

1 Thrombin prepared from beef plasma is rapidly inactivated when incubated with human or beef plasma or horse serum.

2 Thrombin is not inactivated when incubated with fibrinogen solution.

3 Inactivation of thrombin by plasma or serum proceeds exponentially, and plasma samples from different normal individuals or from the same individuals over a period of time yield quantitatively similar results.

4 The property of inactivating thrombin is resident in a particular fraction of plasma albumin which is capable of being concentrated and may be enzymatic in nature.

The authors wish to express their gratitude to Drs E. J. Cohn and T. L. McMeekin for suggestions in this study.

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AL KALOSIS AND LOW PLASMA POTASSIUM IN A CASE OF CUSHING'S SYNDROME: A METABOLIC STUDY

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Addison's disease is characterized to a large degree by pathologic changes in the metabolism of electrolytes but changes in the sexual organs and sexual characteristics either do not occur or are inconspicuous. In cases of Cushing's syndrome¹ on the other hand demonstrable abnormalities in the metabolism of electrolytes occur only exceptionally but changes in the sexual organs and sexual characteristics are the rule. In both diseases amenorrhea or impotence may occur although neither symptom occurs with regularity in cases of Addison's disease. In view of the fact that hyperplastic or neoplastic changes occur in the adrenal cortex with considerable regularity among cases of Cushing's syndrome it is indeed remarkable that abnormalities of electrolyte metabolism occur so infrequently. In a series of more than thirty cases thirteen of which were adrenal cortical tumor we found only three cases in which there were marked changes in the electrolyte pattern of the blood.² The first case of this type was reported by one of us in 1933 (1).

¹ The term *Cushing's syndrome* is used here in the sense suggested by Kessel and refers to the clinical picture described by Cushing namely hirsutism amenorrhea or impotence plethoric obesity purplish striae, hypertension and osteoporosis. Cushing's syndrome has been found in association with basophilic tumors of the anterior pituitary body neoplasms of the adrenal cortex and hyperplastic lesions of the adrenal cortices (sometimes in conjunction with thymomas). Cases have also been described in which necropsy findings have been extremely meager. In nearly all instances Crooke's hyaline changes of the basophilic cells of the anterior pituitary body can be found. The term *Cushing's disease* refers to Cushing's syndrome that has occurred in association with a basophilic adenoma of the pituitary body.

² In some of the earlier cases such changes were not sought with the care now possible as the result of improved chemical methods. Nevertheless it is unlikely that such electrolyte changes were present, as the clinical picture that follows the disruption of the chemical constituents of the blood is as striking as the changes that occur when a patient suffering from Addison's disease experiences a "crisis."

prior to our knowledge that the adrenal cortex was related to the metabolism of electrolytes. A few years later we published the findings concerning a second case (2, 3).³ This report deals with our third case.

In 1937 McQuarrie, Johnson and Ziegler (4) studied and reported the electrolyte changes that occurred in another case. In all of these cases there was a severe alkalosis associated with a low concentration of chlorides in the plasma and in McQuarrie's case as well as in the present one the concentration of potassium in the plasma was found to be low. (The plasma was not analyzed for potassium in the first two cases.) The occurrence of a low concentration of chlorides in the plasma in Cushing's syndrome has been a stumbling block in understanding the pathologic chemistry of this condition because in contrast to Addison's disease one might expect these chlorides to be increased or at least normal.

The present study was undertaken in order to obtain more data as to the nature of the chemical changes that are associated with this syndrome.

Throughout the study the patient received a weighed diet low in content of sodium chloride and moderately high in potassium (approximately 40 grams of potassium 0.6 gram of sodium and 10 gram of chloride) such as is used in the provocative test for the diagnosis of Addison's disease (6, 7). Additional salts (potassium citrate, potassium chloride or ammonium chloride) were administered in solution in divided doses during waking hours or in the form of an 0.8 per cent

³ In both case reports alkalosis was mentioned but was not stressed. In the second case reported, death ultimately occurred and the findings at necropsy were reported by Norris (5). He felt that the primary lesion was an arrhenoblastoma of the ovary from which metastasis had occurred. This explanation scarcely accounted adequately for the events that took place during the patient's illness and was questioned by two competent histopathologists who subsequently examined sections of the tumor and the metastatic lesions.

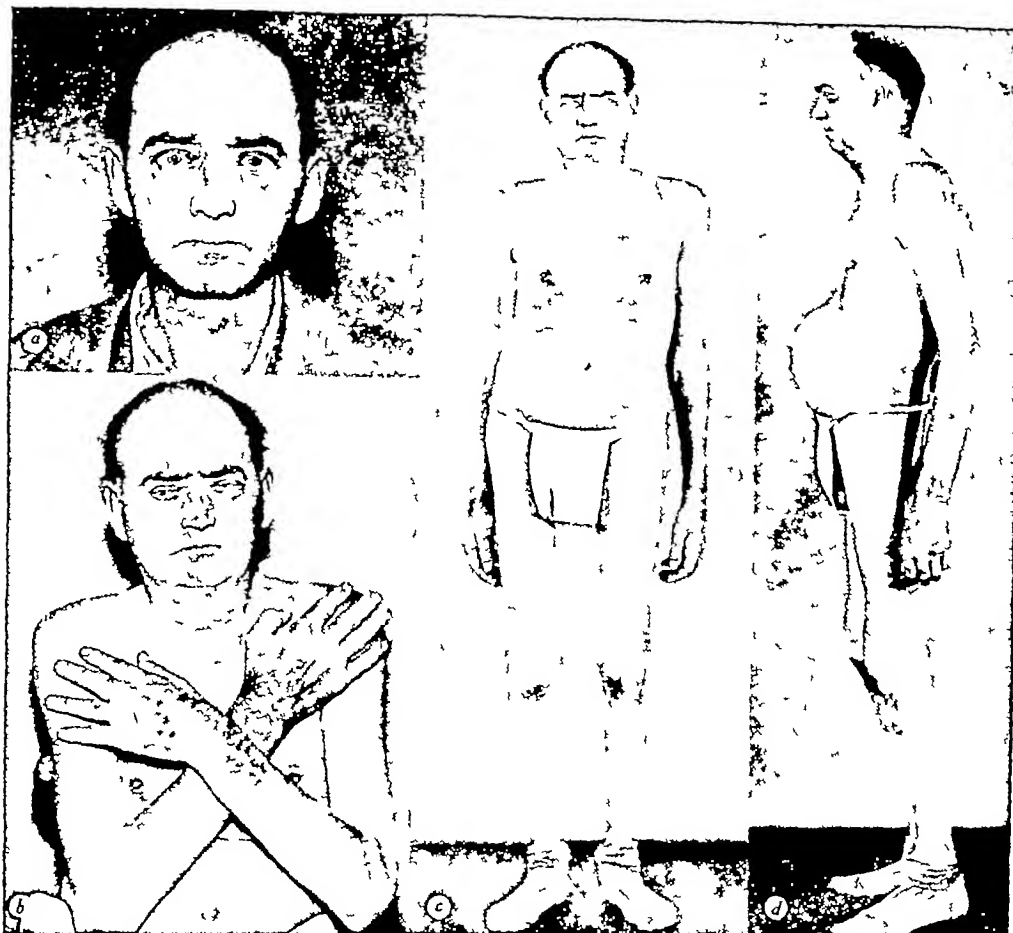


FIG 1 APPEARANCE OF PATIENT

(a) October 17, 1938, (b), (c), and (d) August 28, 1939

solution intravenously. Specimens of urine for each period of twenty-four hours were collected and were preserved with thymol in the refrigerator and aliquot portions were analyzed. Feces were separated into five- or seven-day periods by means of carmine markers, and aliquots were also analyzed for mineral components.

In the morning before the administration of food or salt solution, blood was collected under oil without the use of a tourniquet in order to avoid stasis. Heparin was used as an anticoagulant. The specimens were chilled and analyses were begun immediately on the plasma.

CHEMICAL METHODS

The carbon dioxide content of the plasma as separated, and of another sample equilibrated at 38° C at a carbon dioxide tension chosen as closely as possible to that of separated plasma was determined by the method of Van Slyke and Neill (8). From these data the pH of the

plasma as separated was calculated according to the method of Eisenman (9).

Chloride in plasma and in homogenized suspensions of feces was determined by a slight modification of the method of Keys (10), that in urine by the Volhard-Harvey titration with the reagents described originally for plasma by Wilson and Ball (11). Sodium in plasma and urine was determined by the method of Butler and Tuthill as quoted by Peters and Van Slyke (12a), and potassium by the chloroplatinate method of Shohl and Bennett as modified by Hartzler (13). Feces were dried and powdered and weighed samples were ashed in an electric muffle overnight at 500° C, after preliminary digestion with sulfuric acid. The ash was extracted with 0.5 normal hydrochloric acid and was analyzed for sodium and potassium by the methods cited.

The total nitrogen content of plasma, urine and homogenized suspensions of feces was determined by a modified Kjeldahl procedure.

REPORT OF CASE

A white, male farmer, aged thirty-nine years, first came to The Mayo Clinic on October 5, 1938, because

of weakness, loss of weight and mental depression. The illness gradually developed during a period of six months and was punctuated by two episodes of transient painful edema of the face and lower extremities associated with paresthesias of the legs and fingers. Two months prior to admission diabetes and hypertension were first noted, and mental apathy frequent spells of crying and failure of memory became noticeable. Twenty five pounds (11.3 kgm.) were lost in the period of four weeks which preceded his coming to the clinic. In addition, there was a loss of libido of several months' duration. The use of alkalis or drugs was denied, and there had been no vomiting.

Physical examination revealed a negativistic, confused, dehydrated individual who appeared fifteen years older than his stated age and obviously was ill. He weighed 118 pounds (53.6 kgm.) The blood pressure was 190 mm. of mercury systolic and 110 mm. diastolic. The temperature and respirations were normal and emphysema was not present. Some suggestion of a moon face was evident, but there was comparatively little of the characteristic obesity or plethora of Cushing's syndrome (Figure 1a) There was no change in the secondary sexual characteristics, the breasts and testes appeared

normal to palpation. Neurologic examination gave negative results, the cerebrospinal fluid contained 60 mgm. of protein per 100 cc. The basal metabolic rate was +16 per cent. The ocular fundi and visual fields were normal, as were also the excretory urogram and roentgenograms of the head. Roentgenoscopy failed to reveal any abnormality of the thymus.

The fasting blood sugar on admission was 173 mgm. per 100 cc. The blood sugar at 11 a.m. and 4 p.m. was 278 mgm. and 312 mgm. per 100 cc., respectively. The urine was usually sugar free in the morning but moderate to severe glycosuria without ketonuria was present in the afternoon and evening. The daily administration of 20 units of protamine insulin practically eliminated the glycosuria. During the first six days of hospitalization prior to the observations to be reported (October 5 through 11 1938) a marked negative fluid balance was present with urinary volumes averaging 4500 cc. daily on an intake averaging 3500 cc. This loss of fluid occurred even though glycosuria had been minimized by the use of insulin.

The most striking abnormalities observed were severe alkalosis (without signs of tetany) and the decrease in concentration of potassium in the plasma (Figure 2d)

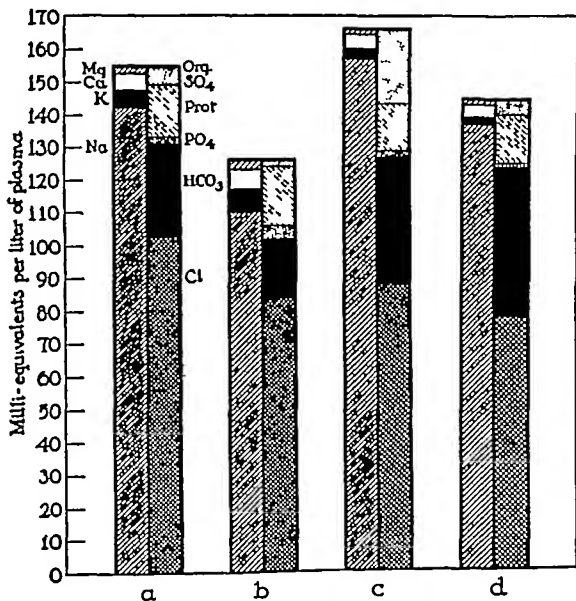


FIG. 2. PATTERN OF ELECTROLYTES

(a) of a normal individual, (b) in an Addisonian crisis, (c) in a case of "hypercortico-adrenalism" and (d) in our own case of "adrenalism" herein reported (columns a b and c are from McQuarrie (

concentrations of blood sugar were at 8:00 a.m. (fasting) 101 mgm., at 11:30 a.m. 163 mgm., and at 4:00 p.m. 157 mgm. per cent. The tourniquet test (at 130 mm. mercury for ten minutes) gave negative results. The basal metabolic rate was -3 per cent, the sedimentation rate was normal and roentgenoscopy of the thorax again gave negative results. The electrolyte pattern of the plasma was again deranged (Table I), although not to the degree observed at the initial admission. At this time, the pH of the venous plasma, determined gasometrically, was found to be 7.54. This confirmed the previous impression that a state of uncompensated alkalosis existed.

Inasmuch as the results of the previous study had indicated that administration of potassium and chloride salts would correct the abnormal chemical composition of the blood, and that potassium chloride would maintain this correction, an effort was made to determine whether an excess of potassium or chloride ions individually would have a similar beneficial effect. Accordingly the patient again received the basal diet previously described (period 4). The sodium chloride was increased, however to approximately 6 grams daily (period 5). The concentration of electrolytes in the plasma remained essentially unchanged during these two control periods, except for a slight increase in the concentration of potassium which was accompanied by a slight retention of potassium. A negative balance for sodium and chloride in period 4 occurred as one might expect, in a short period of sodium chloride restriction and the compensatory positive balance followed a return to an average intake of sodium chloride in period 5.

The addition of 17.5 grams of potassium citrate per day to the diet (period 6) was associated with retention of a large amount of potassium, and the concentration of potassium in the plasma again increased. Coincidentally, there was a small but definite increase in the concentration of chloride in the plasma and a decrease in the concentration of bicarbonate in the plasma from the levels maintained during the two previous periods. This occurred in spite of the fact that no additional chloride ion was administered.

The substitution of 6 grams of ammonium chloride daily for the potassium citrate (period 7) resulted at first in a retention of chloride with a further increase in its concentration of the plasma and a decrease in concentration of bicarbonate. At the same time, however a negative balance for potassium was observed and the concentration of the plasma potassium decreased to very low levels. Thereafter in spite of continued administration of this same amount of ammonium chloride (period 8) the trend toward increasing concentrations of bicarbonate and decreasing concentrations of chloride in the plasma persisted. It would appear therefore, that ammonium chloride was effective in the elevation of the concentration of chloride (and decrease in bicarbonate) in the plasma only when the concentration of potassium in the plasma was maintained near normal levels by the previous administration of potassium citrate.

The patient was observed at intervals following the

completion of these observations and the electrolyte pattern remained reasonably well controlled (Table I) by the administration of 2 teaspoonfuls of potassium chloride daily. He finally discontinued this medication because of gastro-intestinal symptoms, however, and the final analysis of the blood on August 30 1939 revealed a derangement of the electrolyte pattern that was nearly as severe as that which was present at the original admission.

The development of the classical symptoms and signs of Cushing's syndrome continued in spite of four courses of roentgen therapy to the thymus and pituitary glands. The characteristic plethoric moon face, adiposity and protuberant abdomen were present. In addition, osteoporosis and compression fractures of the spine developed and the patient decreased 10 cm. in height within one year. A productive cough and paralysis of the left vocal cord, which developed following completion of the metabolic study were suggestive of the possibility that a mediastinal lesion was present but this could not be confirmed.

Finally weakness and cachexia developed (Figure 1 b c and d) the diabetes became more severe, and the patient became bedfast and finally succumbed eighteen months after the onset of symptoms. Death occurred elsewhere and necropsy was not performed.

COMMENT

The striking abnormalities in the electrolyte pattern of the plasma of this patient consisted of an elevated concentration of bicarbonate, and a decreased concentration of chloride and potassium. In considering the bicarbonate-chloride relationship, the usual causes for such a disturbance were not present in this case. The possibility of a primary bicarbonate excess of respiratory origin is excluded by the observed pH of 7.54 (12b) and alkalosis resulting from administration of alkali or from loss of chloride by vomiting is likewise excluded. Apparently, the patient's condition represented an acid-deficient type of alkalosis of unusual origin.

The metabolic data indicate that, in addition to the persistently low value for potassium in the plasma, the intracellular stores of potassium were probably greatly depleted. During the initial seven-day period of study the concentration of potassium in the plasma increased from 2.2 to 3.5 milliequivalents per liter. Such an increase would require, if equally distributed in the total body water (calculated as 70 per cent of the body weight), a retention of only about 2 grams of potassium. From the metabolic balance however, it may be seen that 25.8 grams of potassium were retained, or (allowing for

through the skin) approximately ten times the amount calculated necessary to produce the observed increase in concentration in the extracellular water. This is in decided contrast to the small amount of potassium required to restore an equally diminished concentration of potassium in the plasma to normal in two cases of obstructing lesions of the colon in which treatment was by prolonged nasal suction with the Miller-Abbott tube.⁴ The recurrence of depletion of potassium in our case seems to be compatible with the results of recent investigations (14 to 19), which have shown that adrenal cortical extracts (and desoxycorticosterone) augment the urinary excretion of potassium when administered to normal or adrenalectomized animals or to patients who have Addison's disease.

The presence of a low concentration of chloride in the plasma is less easily understood. That it may in some way be conditioned by potassium, as suggested by McQuarrie (4), would seem to be supported by the fact that the concentration of both chloride and potassium in the plasma increased following the addition of potassium citrate to the diet, and by the failure of ammonium chloride alone to exert a beneficial effect on the concentration of chloride in the plasma, except when the concentration of potassium was normal. In this connection, it is of interest to note that the electrolyte pattern of the plasma of rats maintained on a diet deficient in potassium is in many respects similar to that seen in our case, that is, sodium relatively normal, but both chloride and potassium markedly decreased (20).

The absence of marked abnormalities in the concentration of sodium in the plasma or of changes in the metabolic balance of sodium is compatible with the picture of hypercortico-adrenalism. The formation of edema following the administration of large amounts of sodium salts or by the use of desoxycorticosterone in Addison's disease suggests that a moderate increase in the content of sodium within the body may be compensated for by an associated retention of water and thus may not be reflected by an increase in the concentration of this ion in the plasma. That an increase in the concentration of sodium

in the plasma may occur, however, was demonstrated in the initial analyses of the plasma in the case reported by McQuarrie and his co-workers and this increase represents the major difference in the electrolyte pattern in these two cases (Figure 2).

It would appear from these observations that the hypothesis of hypercortical adrenalism in our case is tenable and that this hyperfunction was responsible for a loss of potassium from the body.

SUMMARY

A typical case of Cushing's syndrome was studied because of the association of a disturbance in the electrolytes of the plasma, namely, diminished concentration of potassium and chloride, increased concentration of bicarbonate, elevated pH, and normal concentrations of sodium, protein, calcium, magnesium, phosphorus and sulfates, and undetermined acid fraction.

The pattern of the plasma electrolytes returned to normal following the simultaneous administration of potassium chloride and ammonium chloride. Thereafter, the administration of potassium chloride alone maintained a relatively normal pattern.

The administration of potassium citrate was followed by partial correction of the abnormal concentrations of potassium, chloride and bicarbonate in the plasma.

Ammonium chloride failed to maintain a normal concentration of plasma chloride and bicarbonate after the concentration of plasma potassium decreased to low levels following discontinuance of administration of potassium salts.

The concentrations of other electrolytes in the plasma remained essentially normal throughout the period of observation and showed no apparent relation to the metabolic upset.

It is suggested that the abnormality in the distribution of electrolytes in this case is the result of the failure of the kidney to retain potassium, and that the chloride plays only a secondary rôle as it appears to do in cases of adrenal insufficiency.

The therapeutic administration of potassium chloride to this patient did not influence the course of the disease.

⁴ Unreported cases.

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EFFECTIVENESS OF PER-ORAL INSULIN IN HUMAN DIABETES

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In previous reports one of us (M) and his associates (1, 2) have shown that hexylresorcinol aids in the absorption of insulin from the gastrointestinal tract of dogs. It has since been shown that other derivatives, the octyl and heptyl resorcinols (3) and the pentyl, butyl, and propyl resorcinols likewise are active in this physiologic process, the last two, however, less so than the higher compounds. Ethyl resorcinol proved completely inactive, as have the acyl resorcinols and the butyl ester of β resorcinol (4). Reports on the effects of other substances found to retard digestion of insulin will be made in later papers.

None of the resorcinols having proved superior to the hexyl compound, it was decided to try this one on a series of diabetic patients.¹

At the time of the report before the National Academy of Sciences (5) in Rochester (October 1937), it was stated that, "Trials with human subjects (normals) permit us at the present time only to state with certainty that, under the conditions defined, insulin can be absorbed in sufficient amount to produce abrupt changes in blood sugar. With some subjects we have thus far not been able to demonstrate any absorption and, under certain conditions not yet understood, persons who have previously given good absorption fail to do so. It is impossible to say at the present time how useful or important these observations may become in the treatment of diabetes."

With this statement plainly before him, a certain reporter sent to the Associated Press a dispatch completely at variance with the above, and the erroneous report was widely copied in the lay press. The story grew to the point where "Time" reported "a perfected compound of insulin and hexylresorcinol which may be swallowed as a tablet." Not a word was said about tablets either in the paper read before the Academy, the abstract published in Science, or in the statement given to the press along with statements from other speakers.

Trials of the absorption mixture which had

proved most effective in animal experiments were begun with diabetic patients in October, 1937 and were continued without interruption until June, 1938. They were resumed in December, 1938 and continued until July 1939. Twenty patients were given the oral insulin over periods varying from two weeks to three and a half months. The results are reported in summary with some details on patients who showed greatest responses. It was not anticipated from the experience gained with dogs that it would be possible to replace all subcutaneous insulin in any other than the mildest cases requiring insulin. The report should not be regarded as anything more than a physiologic study of absorption of insulin from the alimentary tract of patients who have an interest in the outcome. Its value from the standpoint of treatment should be appraised in several other clinics where dietary and other factors can be controlled rigidly before there can be any hope of general usage by the medical profession. Improvement of absorption by other adjuvant substances has been obtained recently in the animal laboratory, but the point of safe application to the diabetic patient has not yet been reached.

Of the twenty patients selected from the outpatient diabetic clinic or the inpatients of Strong Memorial, Municipal and General Hospitals in Rochester, two were studied in a preliminary way without an adequately controlled diet, four proved uncooperative, two became sugar free without insulin, one was quite old (68) and gave very little response to oral insulin and one of the two who thought they had to have protamine insulin was very difficult to regulate to a satisfactory base level of sugar excretion or fasting blood sugar. Of the twenty only twelve gave good evidence of absorption under conditions which cannot be impeached.

These conditions included (1) a period of adjustment of subcutaneous insulin to a point where both change of sugar excretion and change of blood sugar could be reliably measured (2) per-

¹ This investigation has been aided by a grant from the Insulin Committee of the University of Toronto contributed by the manufacturers of insulin in the U. S. A.

TABLE I

Total digestibility not affected by oral insulin + hexylresorcinol and buffer
(Subject T L)

Date 1938	Period			N	Protein	Fat	Carbo hydrate	Ash
	Number	Days						
January 4-8	I Control	5	Food	grams 14 53	grams 90 8	grams 79 88	grams 142 2	grams 14 57
			Urine	11 96				
			Feces	1 16	7 2	3 53		4 41
			Balance	+1 41	83 6	76 35		10 16
			Apparent digestibility, <i>per cent</i>		(92 0)	(95 5)		(69 7)
January 9-15	II 1 to 4 doses 100 to 400 units insulin, 0 125 to 0 50 gram hexyl- resorcinol, pH 10 5	7	Food	14 53	90 8	79 9	142 2	14 57
			Urine	13 27				
			Feces	1 33	8 3	2 7	1 9	4 48
			Balance	-0 07	82 5	77 2	140 3	10 09
			Apparent digestibility, <i>per cent</i>		(90 8)	(96 6)	(98 6)	(69 2)
January 16-24	III Capsules 400 units in- sulin + 0 5 gram hexylresorcinol 4 None pH 10 5	9 5	Food	14 53	90 8	79 9	142 2	14 57
			Urine	12 92				
			Feces	1 29	8 1	2 3	1 1	4 26
			Balance	+0 32	82 7	77 6	141 1	10 31
			Apparent digestibility, <i>per cent</i>		(91 0)	(97 1)	(99 2)	(70 7)
January 25-30	IV 4 doses 800 units in- sulin + 1 0 gram hexylresorcinol, pH 10 5	6	Food	14 53	90 8	79 9	142 2	14 57
			Urine	13 91				
			Feces	1 54	9 6	2 2	1 3	5 22
			Balance	-0 92	81 2	77 7	140 9	9 35
			Apparent digestibility, <i>per cent</i>		(89 4)	(97 2)	(99 0)	(64 1)

(Subject M D)

January 4-8	I Control	5	Food	11 59	72 4	73 1	136 0	11 48
			Urine	10 98				
			Feces	1 04	6 5	8 3	2 7	
			Balance	-0 43	65 9	64 8	133 3	
			Apparent digestibility, <i>per cent</i>		(91 0)	(88 6)	(98 0)	
January 9-15	II 1 to 3 doses daily 100 to 300 units insulin, 0 125 to 0 375 gram hexylresorcinol, pH 10 5	7	Food	13 84	86 5	77 6	131 9	12 26
			Urine	10 99				
			Feces	1 12	7 0	2 4	3 0	1 32
			Balance	+1 73	79 5	75 2	128 9	10 94
			Apparent digestibility, <i>per cent</i>		(91 9)	(96 9)	(97 9)	(89 2)
January 16-24	III Capsules, 4 No oral, 5	9 5	Food	13 84	86 5	77 6	131 9	12 26
			Urine	10 71				
			Feces	0 96	6 3	2 6	3 4	1 05
			Balance	+2 17	80 2	75 0	128 5	11 21
			Apparent digestibility, <i>per cent</i>		(92 7)	(96 6)	(97 4)	(91 4)
January 25-30	IV 4 doses daily 800 units insulin, 1 0 gram hexylresorcinol, pH 10 5	6	Food	13 84	86 5	77 6	131 9	12 26
			Urine	12 54				
			Feces	1 21	7 6	2 4	4 3	0 96
			Balance	-0 11	78 9	75 2	127 6	11 30
			Apparent digestibility, <i>per cent</i>		(91 2)	(96 9)	(96 7)	(92 1)

fectly constant diets during the adjustment period and subsequent experimental periods, (3) strict confinement in the hospital under careful surveillance of a competent special nurse.

Effect on digestion

At the beginning of this work it was felt that the degree of interference, if any, of the controlling substances with total digestion must be known in order that a proper assay of the effectiveness of the insulin might be given. If, for example, the high pH of the mixture or the hexylresorcinol should interfere materially with digestion or absorption of carbohydrate or protein, a diminution in the excretion of sugar might be attributed erroneously to the oral insulin.

Two subjects therefore, were placed on the rigidly controlled diet. A fecal marker was given with the first breakfast in each period and another with the first breakfast following the experiment. Feces were collected from the first appearance of one mark to the first appearance of the next. There was an occasional loose stool, but with only one exception this occurred after a period was well started and in the single instance mentioned no difficulty was found in making satisfactory separation.

Precisely one half the day's diet on two separate days was weighed out, in addition to the amount served, and this half-portion was dried down for analysis. Food and feces were analyzed by identical methods for total nitrogen, total fat (ether extract), total ash and total hydrolyzable sugar, expressed as glucose. Total carbohydrate was also found in the food by subtraction. Twenty-four hour urines were collected and analyzed for total nitrogen and total sugar.

In Table I, the resulting figures are given as well as the N balance and the apparent digestibility expressed as percentages of each nutrient fed. The term "apparent digestibility" is used as the equivalent of the older term "coefficient of utilization" or "coefficient of absorption." It gives recognition to the fact that there is an endogenous excretion of nitrogen from the gut (now called alimentary nitrogen) which should be subtracted from fecal nitrogen to give the true food nitrogen remaining unabsorbed. The apparent digestibility serves equally well for the pur-

pose in hand. No material retardation of digestion or absorption can escape, and to obtain true digestibility would require at least one period of several days without any protein in the diet, a procedure which we hesitated to impose on a diabetic.

The periods for subjects T. L. and M. D. coincided. Period I, of five days' duration, was a control, no oral insulin being given. In Period II from one to three standard doses were given, each consisting of 200 cc of water in which were dissolved 100 units of insulin, 0.125 per cent of hexylresorcinol, and a mixture of sodium carbonate and bicarbonate sufficient to give a pH of 10 to 10.5. Period III started out to be one in which the same quantity of insulin and adjuvant substances in dry form for each dose would be taken in a gelatin capsule. These capsules were not successful and produced nausea, consequently, they were discontinued at the end of four days. The period continued however, for five days longer without any insulin. Then followed Period IV during which for six days a double dose (200 units plus corresponding amounts of hexylresorcinol and sodium salts) was given four times each day, making a total of 800 clinical units and 10 gram hexylresorcinol.

It is readily seen (Table I) that no material difference in the apparent digestibility of the protein, fat or carbohydrate occurred as between the period with no insulin and either of those with insulin and the adjuvant chemicals. Changes equally great occur in successive periods on any weighed and analyzed diet which is constant. There is however with one exception a slightly greater output of nitrogen in the full oral insulin periods. Since this concerns the urine principally, it could not be attributed to lessened digestion but rather to faulty retention.

The only change which is consistent throughout all the comparisons is a *slightly* better absorption of fat when the hexylresorcinol was given. The difference in the first comparison with subject T. L. was only 1 per cent, but with subject M. D. it was over 8 per cent. The better absorption of fat is readily explained by the emulsifying action of hexylresorcinol, this same property (surface tension lowering) possibly affecting also the absorption of insulin. Carbohydrate (expressed as

hydrolyzed glucose), the component of the diet about which we were especially concerned, shows no material change in absorption either way. The absorption of the ash was rather low in subject T L—notably so in the last period. But no consistent adverse effect of the oral mixture is to be made out. Two other subjects studied for a shorter time in the same manner gave the same evidence of non-interference with digestion.

Naturally these findings were reassuring and we have not hesitated since then to give up to four double doses of the insulin-hexylresorcinol-alkaline salts mixture (800 I U) each day. There have been no signs of interference with digestion and very little interference with appetite (see H G, Table II) in any of the subjects so long as the solutions described were used. Gelatin capsules containing hexylresorcinol in dry form sometimes have produced nausea and in certain instances, when sufficient water has not been taken, they have been regurgitated. Enteric coated tablets containing insulin and the adjuvant materials have not as yet been proved to have any comparable effect. The results described below apply, with certain exceptions noted, only to the effects of insulin given in solutions.

PROCEDURE

Each patient on admission was placed on a controlled diet, estimated to be sufficient to maintain body weight, with some deficiency of insulin, in the ambulatory hospital life he was expected to lead. More than half the patients were bedded in the regular medical divisions of Strong Memorial Hospital, but five of the twelve and some others were cared for in the metabolism unit of the Department of Medicine where facilities for close observation of the patient and feeding from the metabolism kitchen were available. At times patients in the regular divisions were given their meals in the Department of Vital Economics from its own diet kitchen. This required only a walk up one flight and into an adjoining wing of the medical school. No patient was allowed to leave the hospital during an experiment or to leave the prescribed limits without permission in each instance. A recreation room was fitted up in the Department of Vital Economics where men patients bedded in the divisions could listen to the radio, smoke, and play games. In the metabolism unit, also, radio receiving sets were provided in the rooms and smoking was permitted so long as the patient remained strictly in his room.

The nurse in charge of the cases in the division or metabolism unit, as the case might be, exercised watchful care over the collections of urines in definite periods, the

administration of subcutaneous insulin, *etc* at all times. When it was necessary to give subcutaneous insulin at night to patients in the metabolism unit, this was done by a night nurse from an adjoining division of the hospital. Meal times were kept regular, and a special dietitian prepared the food, weighed all food served, and weighed back anything left on the plate. Diets were kept as nearly constant as the variations from standard food tables would permit. The task of analyzing all foods consumed was too great to be undertaken. Reliance was placed on long periods, or repetition a sufficient number of times, to equalize variations and give dependable averages.

Oral insulin works best on an empty stomach, and with the idea that two meals a day, without change of total or distributed calories, might afford greater chance of absorption, this alteration was made for a short time with two patients (P J and W V), but there was no greater total effect (see W V, Table II). The two-meal method with oral insulin a short time before each meal would probably have given greater results with some of the more responsive subjects.

In the adjustment of subcutaneous insulin to a level which would permit excretion of sufficient sugar for reliable determinations both qualitatively and quantitatively, it was, of course, necessary to proceed with caution both as regards the total insulin given and the distribution. The requirement of a hospitalized patient decreases considerably below that of the patient at home, in most cases, and the danger of insulin shock is correspondingly increased. The number of times shock occurred was very small. The greater difficulty arose from the change from protamine-zinc insulin to regular insulin, and in two cases (H C and T V) it was necessary to continue the protamine-zinc variety throughout. If the work were being planned again, it is believed that the use of protamine-zinc insulin might be made the basis of a more accurate confirmatory assay from the fasting blood sugar levels (See section on Unitage Effects below). From early experiments, however, it was found, as expected, that readjustments of the subcutaneous insulin could be made much more rapidly with regular insulin, and this time factor seemed all important. It was known also that some patients cannot tolerate the protamine-zinc mixture. Hence, for uniformity and dependability of results, the use of regular insulin whenever possible was decided upon.

The insulin employed for oral administration also was regular insulin, generously contributed in the form of highest purity powders or crystals by the manufacturers. The administration of the oral doses was always supervised by one of the authors. From experiments on themselves and other normal volunteers, as well as from experiments on dogs (1, 2, 3), they knew that the insulin must be mixed with the hexylresorcinol-buffer mixture immediately before administration to be sure of its survival long enough for absorption. Data on the survival of insulin under various conditions pertinent to this study will be found in the earlier publications. Aqueous solutions of the dry insulin of varying unitage were pre-

pared, based on the assays supplied by the manufacturers and, in many instances, on confirmatory assays on rabbits, made in our laboratories. As in the dog experiments (3) the "standard dose" came to be 100 I U insulin dissolved in a solution of 0.125 per cent hexylresorcinol and sufficient buffer mixture to give a final pH of 10 to 10.5 the total volume being 200 cc. Where a "double dose" is mentioned, it means two standard doses. If the volume were increased for any reason the other solutes were increased correspondingly. Trials were made at times of 0.25 per cent solution of hexylresorcinol but with no better results. In this work on human diabetics no mixture was given which had not already been proved potent in the animal work.

From experiments on normal subjects it was thought for a time that solutions in 10 to 20 per cent alcohol instead of water were more effective and these were tried in a few instances on the human cases, with their consent, but there was no improvement. Repetition on normals completed the disillusionment. Various flavor

ing substances also were used to mitigate the rather biting, astringent effect of hexylresorcinol in the mouth which to some subjects was unpleasant.

Urine and blood

Urines were collected every day in four periods 7 to 11 a.m., 11 to 4 p.m., 4 to 9 p.m., and 9 p.m. to 7 a.m. The conventional eight drops were used for qualitative tests in all these fractions according to the Benedict method, and the results recorded as 0 + 1 + 2 + 3 + and 4 +. These checks proved invaluable in adjusting the daily distribution of subcutaneous insulin as well as in gauging the effects of oral dosage.

In the quantitative determination of glucose in the urine, the copper iodometric method of Shaffer and Somogyi (6) was used after treatment with the HgSO_4 , BaCO_3 reagent devised by West, Scharles, and Peterson (7) for removal of interfering substances.

As soon as the patient began to show a reasonably constant level of urine sugar bloods were drawn by one

TABLE II
Effect of oral insulin on urine sugar and on fasting blood sugar

Patient	Period	Days	Date	Protein	Fat	Carbohydrate	Total calories	Average body weight	Units subcutaneous insulin	Units oral insulin	Urine sugar	Reduction in urine sugar	Change in blood sugar between 7:15 and 8 a.m.	Effect of oral insulin on blood sugar
T. L.	I	3 cont.	January 17-January 19	80	100	200	2075	70.8	35	600*	17.7	-10.9	-22	-100.0
	II	5 cont.	January 20-January 24	80	100	200	2075	70.0	35	None	28.6	+7.8*	-13	-29.0
	III	6 cont.	January 25-January 30	80	100	200	2075	69.5	20	800*	55.0	-50.6	+14	-29.0
	IV	3 cont.	January 31-February 1	80	100	200	2075	68.0	20	None	103.6	-13	+58	-29.0
	V	8 cont.	February 22-March 1	80	123	265	2558	70.1	48	None	15.8	+1.5	-13	-94.0
	VI	10 cont.	March 2-March 11	80	123	265	2558	70.5	43	200-300	17.3	-3.1	+51	-66.0
	VII	9 cont.	March 12-March 20	80	123	265	2558	70.6	43	None	20.4	-1.5	+12	-46.0
	VIII	8 alt.	March 21-April 2	80	123	265	2558	70.8	43	600	19.1	-1.5	+58	-46.0
	IX	5 alt.	March 21-April 2	80	123	265	2558	70.4	43	None	20.6	-1.5	+59	-46.0
	X	5 alt.	June 3-June 14	80	123	265	2558	72.1	48	None	18.5	-10.0	+25	-34.0
	XI	7 alt.	June 3-June 14	80	123	265	2558	71.5	48	300	8.5	-8.5	+1	-25.0
	XII	3 alt.	June 15-June 20	80	123	265	2558	71.4	48	600	22.9	-8.5	+25	-25.0
	XIII	3 alt.	June 15-June 20	80	123	265	2558	70.9	48	None	31.4	-12.5	+33.0	-33.0
M. D.	I	4 cont.	January 16-January 19	65	90	182	1850	62.6	30	450*	14.1	+3.7	0	-8.0
	II	4 cont.	January 21-January 24	65	90	182	1850	61.7	30	None	10.4	-4.4	+8	-18.0
	III	5 cont.	January 26-January 30	65	90	182	1850	61.0	15	800*	40.5	-4.4	+11	-18.0
	IV	3 cont.	January 31-February 2	65	90	182	1850	60.3	15	None	44.9	-0.8	+13.5	-13.5
H. G.	I	5 cont.	February 25-March 1	76	105	210	2150	74.5	44	None	20.0	-7.0	+50	-24.0
	II	12 cont.	March 2-March 13	76	105	210	2150	74.9	44	200-300	13.0	-7.0	+26	-24.0
	III	6 cont.	March 14-March 20	76	105	210	2150	74.3	44	None	19.5	-11.2	+16	-42
	IV	8 alt.	March 21-April 3	76	105	210	2150	74.6	44	600	8.3	-11.2	+16	-42
	V	6 alt.	March 21-April 3	76	105	210	2150	74.2	44	None	25.1	-16.8	+30	-34
	VI	6 cont.	April 26-May 1	76	105	210	2150	74.6	47	None	17.9	-10.2	+24	-32
	VII	5 alt.	May 2-May 11	76	105	210	2150	74.5	47	600*	7.7	-14.9	+40	-48
	VIII	5 alt.	May 2-May 11	76	105	210	2150	74.2	47	None	22.6	-10.3	+34.0	-34.0
J. H.	I	3 cont.	February 27-March 1	75	100	198	2050	72.5	42	None	12.5	-7.2	+52	-30
	II	5 cont.	March 2-March 7	75	100	198	2050	72.7	42	200	5.6	-7.2	+28	-30
	III	14 cont.	March 7-March 20	75	100	198	2050*	71.4	42	None	5.9	-0.3	+58	-31
	IV	5 alt.	March 25-April 3	75	100	198	2050	69.5	36	600	18.4	-6.6	+38	-31
	V	4 alt.	March 25-April 3	75	100	198	2050	69.2	36	None	25.0	-4.7	+31.5	-31.5
H. C.	I	3 alt.	May 1-May 5	107	125	236	2570	56.0	15 P ¹⁰ 10R	None	100.2	-12.1	-4	-1
	II	3 alt.	May 2-May 6	107	125	236	2570	56.0	15 P ¹⁰ 10R	600*	88.1	-12.1	-4	-1
												-12.1	-1	-1.0

TABLE II—Continued

Patient	Period	Days	Date			Protein	Fat	Carbohydrate	Total calories	Average body weight	Units subcutaneous insulin	Units oral insulin	Urine sugar	Reduction in urine sugar	Change in blood sugar between 7 15 and 8 a.m.	Effect of oral insulin on blood sugar
													average grams			
L P	I	4 cont.	June 12-June	15	90	120	238	2460	71.8	15	300-600 None	3.8 14.1	-10.3	-10 + 7	-17	
	II	4 cont.	June 16-June	19	90	120	238	2460	71.3	15						
T V	I	4 alt.	May 8-May	14	82	100	184	2020	39.5	15 P 25 R	600 None	26.6 42.3	-15.7	-15 +13	-28	
	II	3 alt.	May 8-May	14	82	100	184	2020	39.4	15 P 25 R						
F O	I	5 cont.	January 13-January	17	78	107	177	1980	50.0	10	None 100-300 None None 400 800 None 200	12.3 13.6 36.4 51.3 31.9 34.0 15.9 5.6	+ 1.3 -22.8 -19.4 -17.3 -10.3	-10 ¹⁵ -68 -3 -14 -76 -73 -2 -42	-58 -65 -62 -71 -40 ¹⁵	
	II	6 cont.	January 18-January	24	78	107	177	1980	50.1	10						
	III	5 cont.	January 25-January	29	78	107	177	1980	49.4	10						
	IV	2 alt.	January 31&February	2	78	107	177	1980	49.4	10						
	V	5 alt.	January 30-February	5	78	107	177	1980	49.5	10						
	VI	6 cont.	February 6-February	11	78	107	177	1980	49.3	10						
	VII	6 sel.	February 19-February	27	79	110	205	2200	49.9	25						
	VIII	4 sel.	February 23-March	2	79	110	205	2200	49.8	25						
Weighted averages													-17.7		-60	
P F	I	4 cont.	January 14-January	17	69	100	147	1764	65.1	45	None 100-300 None None 400	8.7 4.9 16.0 22.7 11.2	-3.8 -11.1 -11.5	+21 -10 + 8 + 1 -23	-31 -18 -24	
	II	7 cont.	January 18-January	24	69	100	147	1764	65.0	45						
	III	5 cont.	January 25-January	29	69	100	147	1764	64.0	45						
	IV	2 cont.	January 31&February	2	69	100	147	1764	63.9	45						
	V	5 sel.	January 30-February	5	69	100	147	1764	64.0	45						
Weighted averages													-9.1		-24	
P J	I	2 cont.	June 23 & 24		62	80	158	1646	61.2	20	None 200 None 200 None	31.7 17.8 17.0 8.2 19.1	-13.9 -8.8	"		
	II	2 cont.	June 25 & 26		62	80	158	1646	61.5	20						
	III	3 cont.	June 28-June	30	62	80	158	1646	61.4	20						
	IV	3 cont.	July 1-July	3	62	80	158	1646	61.5	20						
	V	3 cont.	July 5-July	7	62	80	158	1646	61.3	15						
Weighted average													-10.8			
E W	I	3 sel.	June 18 20 21 & 22		60	70	135	1230	54.3	40	None 200	13.3 4.4	-8.9	+22 -28	-50	
	II	4 sel.	June 17 19 23 & 24		60	70	135	1230	54.4	40						
W V	I	3 sel.	June 26 27 & 29		70	105	210	2120	70.4	25	None 200 None 400	15.0 8.1 7.7 4.4	-6.9 -3.3	+ 8 -21 -4 -25	-29 -21	
	II	4 sel.	June 28 30 July 1 & 2		70	105	210	2120	70.4	25						
	III	3 sel.	July 7 8 & 12		82	101	217	2120 ¹⁵	69.8	15						
	IV	3 cont.	July 9-July	11	82	101	217	2120 ¹⁵	69.8	15						
Weighted averages													-5.4		-26	

Summary of results of oral insulin in twelve diabetic patients of varying degrees of severity. Diets and subcutaneous insulin held constant in control periods, designated "none" under "Units oral insulin," and periods with varying quantities of oral insulin. This was administered in solution with 0.125 per cent hexylresorcinol and a buffering solution giving a pH to the whole mixture of 10 to 10.5. In third column "cont" means continuous days, "alt." means alternating on and off oral insulin, "sel" means selected days mostly continuous.

¹ Effect of first dose only

² 8 capsules 75 I U each

³ + 2 enteric tablets on 3 days. Refused 250 calories on March 4 and March 5

⁴ 1800 I U thiamin chloride on May 6, May 8 and May 10

⁵ Plus orange juice for reaction on March 23 and March 24

⁶ P = protamine insulin, R = regular insulin

⁷ 3 doses each preceded by 2 capsules containing sodium taurocholate on May 2 and May 4. 4800 I U thiamin chloride on May 6

⁸ Bloods taken on 2 days only

⁹ Sorensen mixture for sake of R-Q

¹⁰ + 2 enteric tablets on 3 days

¹¹ Same since February 6

¹² Three days only

¹³ 6 capsules 75 I U each

¹⁴ Veins too small to get blood

¹⁵ Two meals only at 9 a.m. and 4 p.m.

of the authors every morning soon after 7 a.m., and this procedure was continued throughout each experimental period on or off oral insulin, conditional only on the status of the patient's veins. Sugar was determined by the Shaffer-Somogyi (6) technique after precipitation with the Somogyi (8) zinc-hydroxide reagent.

For gauging the effects of oral insulin on blood sugar, the first dose was given immediately after this first fasting blood was drawn, and a second sample was drawn forty-five minutes to one hour later and fifteen minutes

before the subject's breakfast. The "change in blood sugar between 7 15 and 8 a.m." described in Table II refers to the change from the first to the second of these two samples. The average time was as noted in the heading but, owing to difficulty in getting blood in some patients, there were minor variations in this interval. Frequently, bloods were drawn before and after oral doses taken later in the day, and sometimes the second showed a marked drop in sugar, but as a rule the early morning test gave the greater response.

Results on urine sugar and fasting blood sugar

In Table II will be found complete data on the twelve patients who showed the most positive absorptions. The significant figures are contained in the last three columns but, before discussing those results in detail, a few more words are necessary in explanation of the earlier columns. There were two methods of comparing days with and days without oral insulin. First, a period of several consecutive days on oral insulin as in the first two cases of Table II, followed by several days on the same diet and the same subcutaneous insulin distributed in the same manner. Sometimes the order was reversed as in the second two cases. The dates in Column 4 will enable the reader to see how the two periods were related in time. The second method consisted of alternating successive days, one on the next off oral insulin, with all other conditions identical. The column of dates shows that the two groups of days compared fell in the same total period. All such comparisons are designated "alt" in the table. Sometimes a period which was intended to be continuous had to be interrupted and became alternating or changed otherwise. These are designated "sel," meaning selected days, most of which were continuous.

The figures for grams protein, fat and carbohydrate must not be construed too literally. According to food tables these figures actually were constant, but everyone knows that foods vary from the average figures given in tables in both directions. They should be interpreted therefore, as coming within these variations. The only safeguard aside from actual analysis is always to purchase foods of the same brand or variety. This practice was adhered to very rigidly, thus narrowing the variation as much as possible.

A criterion of adequacy of the diet in relation to the subcutaneous insulin is found in the body weight. For example, it is quite evident that 20 I U of subcutaneous insulin in relation to his diet was not adequate for T L in the third and fourth periods. His weight fell 0.5 kgm in the third period when oral insulin was being taken, but it fell 1.5 kgm in the following period with no oral insulin. The output of sugar in the urine obviously went up for the same reason. The experiment was cut short at that point, and the pa-

tient was given three weeks to become established at a higher insulin level and on a higher diet. He also became more active in this subsequent period.

Subject M D, who was being studied at the same time, exhibits the same picture of inadequacy in Periods III and IV. His weight did not change so much because his subcutaneous insulin was nearer his requirement even at the lower level. Also, he did not derive as much benefit from oral insulin.

Other instances of weight change, though of lesser magnitude as a rule, may be accounted for in the same manner.

The units of insulin in both columns are always international units (I U) and the figures are for twenty-four hours.

In the urine sugar column of figures several features become evident almost at a glance. (1) There is considerable variability intra individually as between different periods with no oral insulin, even when the subcutaneous insulin is constant. (2) The effect of the same total intake of oral insulin is variable for the individual even when diet and subcutaneous insulin remained constant (eg P J). Sometimes however, there is fair agreement (eg H G). (3) Finally, the oral insulin like subcutaneous insulin, has its greatest effect when the initial urine sugar is high (see especially subjects T L and F O) rather than when it is low.

The first type of variability is explained in part by the principle of accumulating deficits. Lack of insulin today should be made up tomorrow. If this does not take place the sugar excretion tomorrow will be still higher than today. The failure of oral insulin in the intervening periods to make up the deficit of subcutaneous insulin obviously leaves the subject behind though not so far behind as if he had had no oral dose. Sometimes the effect of the oral dose is quite significant but of course, very small in relation to the number of units taken. Variability in the effect from the same number of units taken orally might be explained in part also by the accumulated deficits, but in the instance of P J it probably is due in some measure to improvement with rest in the hospital. Twenty units were far from adequate in Period I but helped by the intervening

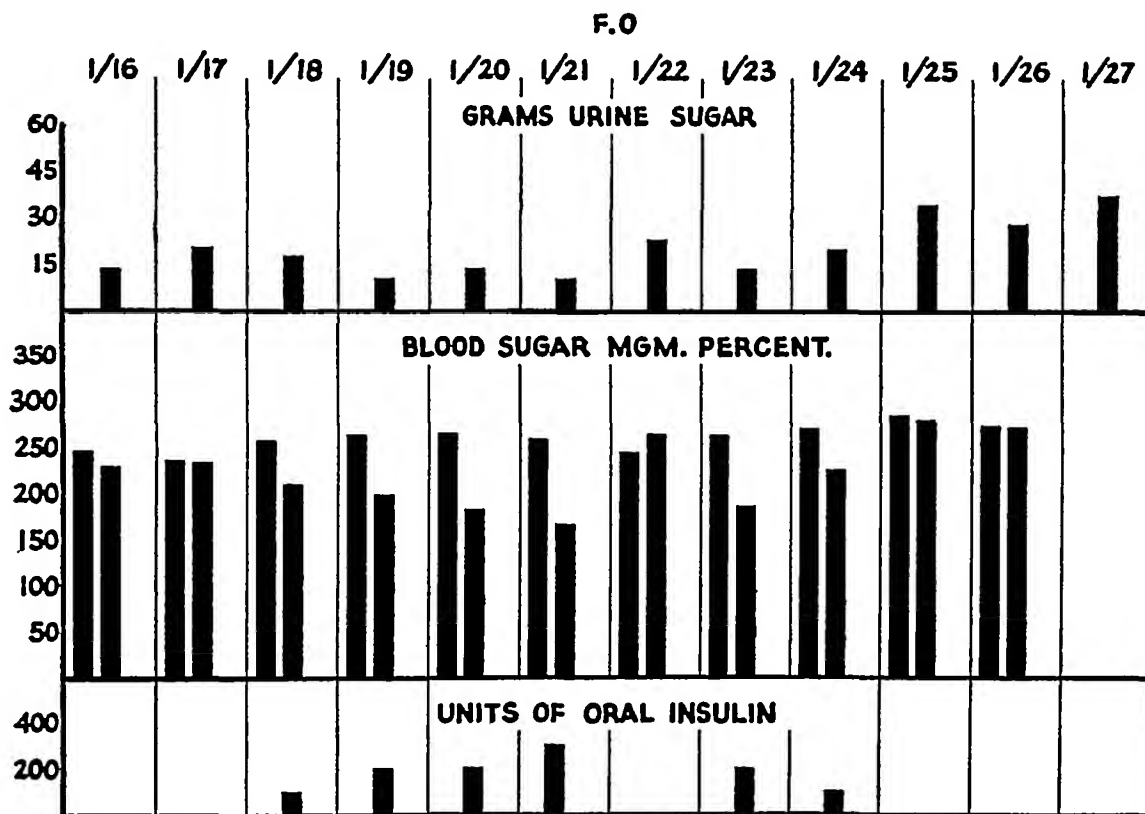


FIG 1 DAILY RECORD OF URINE SUGAR AND BLOOD SUGAR CHANGES FOR PATIENT F O, WITH AND WITHOUT ORAL INSULIN, ALL OTHER CONDITIONS REMAINING CONSTANT

The urine sugar is in 24-hour amounts. The blood sugars shown are first bar, before oral insulin, second bar, 45 minutes after oral insulin, or at corresponding intervals when oral insulin was not given.

200 units of oral insulin, they had become more nearly so in Period III. Food intake was constant, but the patient's condition had changed.

There is, so to speak, a self-limiting consequence of accumulated deficits in the sense that, in terms of sugar saved, the body makes better use of any given dose of insulin when the wastage is great than when it is small. A muscle "knows how" to adapt itself to a load. Why should not the liver exhibit the same "intelligence"?

Weighted averages. The significant figure showing the effect of oral insulin on urine sugar for each individual case is the figure opposite the words "weighted averages." This figure is obtained by multiplying each of the figures indicating change in urine sugar, immediately above, by the number of days on oral insulin which that figure represents. The algebraic sum is then obtained and divided by the total number of days on insulin. In each of the cases listed in this

table, there is a net reduction in urine sugar which is measurable and which, under the circumstances of the experiment (with the exception of M D), is believed to be significant.

The largest effect was obtained with patient F O, who tolerated the oral insulin remarkably well and also gave results which were nearly proportional to the amount of oral insulin administered (see Figure 1). Very good effects were also obtained on patient H G (see Figure 2). These two subjects were the most consistent ones of the entire list in the character of their reactions. Patient T L was longest under the treatment with oral insulin, but his reaction was quite variable, as may be observed from the figures opposite the several oral periods. It is obvious from these results on urine sugars that oral insulin given in the manner described has but little effect on urine sugar, except when the urine sugar is quite high, that, in general, the result is not pro-

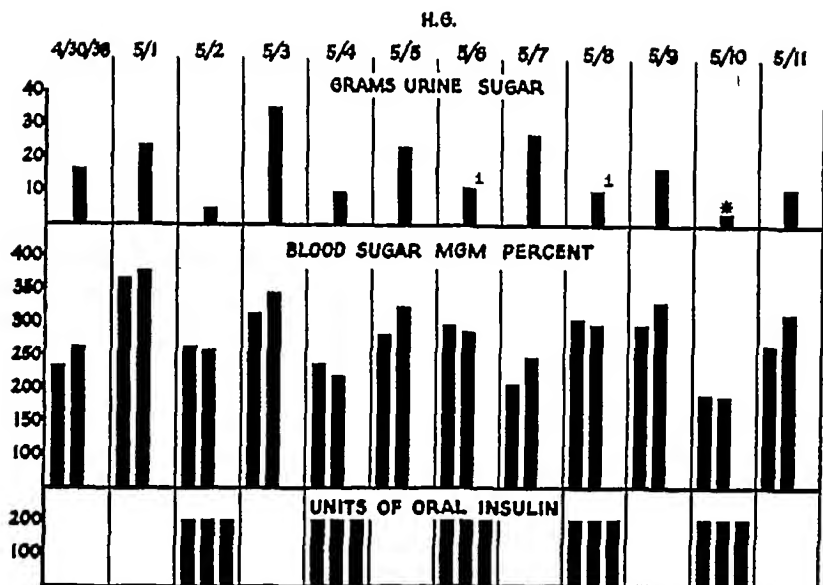


FIG. 2. DAILY RECORD OF URINE SUGAR AND BLOOD SUGAR CHANGES FOR PATIENT H. G., WITH AND WITHOUT ORAL INSULIN. ALL OTHER CONDITIONS REMAINING CONSTANT.

The urine sugar is in 24-hour amounts. The blood sugars shown are first bar before oral insulin, second bar 45 minutes after oral insulin, or at corresponding intervals when oral insulin was not given.

¹ Indicates 1500 I U thiamin chloride with each dose. * Indicates not entire amount of urine sugar.

portional to dosage, and that only very mild cases could hope to replace subcutaneous insulin with oral insulin as here administered.

Change in blood sugar The change in blood sugar from a single dose of oral insulin is at times very striking. For example in the first test with patient T. L., the average effect on the blood sugar of a single dose taken early in the morning was 100 milligrams' reduction, the weighted average for all the tests on this patient was 53 milligrams' net reduction. In most of the tests this patient showed a rising blood sugar in the early morning when oral insulin was not given. When it was given, the fasting blood sugar took a downward turn, actually falling between first and second bloods, or the rise was very much diminished giving in either case a net reduction of considerable magnitude. The same sort of behavior is

illustrated by several other patients. With patient F. O. however, it will be observed that the fasting blood sugar was falling between the two blood samples in the early morning when no oral insulin was given, but when oral insulin was given it fell much farther, and the average reduction with this case was 60 mgm. per cent in the period between the two blood samples. It should be borne in mind that the oral insulin was given immediately after the first fasting blood sugar. There would be no point in giving a grand average net reduction of fasting blood sugar by means of oral insulin for all of the cases. Only one patient, H. C., failed to show a significant drop with the oral insulin and thus may be connected with the circumstance that he was receiving protamine insulin throughout the experiment.

TABLE III
Effect of oral insulin in terms of subcutaneous insulin (regular)

Patient	Average grams urine sugar— subcutaneous test				A Difference in subcu- taneous insulin	B Grams sugar*	Average grams urine sugar— oral test				C Differ- ence due to oral insulin	Equiv- alent† effect in subcu- taneous units
	1st period	Number days	2nd period	Number days			With oral	Number days	Without oral	Number days		
T L	15 15	6	18 4	4	5	0 64	17 6	4	28 1	5	10 5	16 0
T L	28 1	5	105 7	3	15	5 2	45 7	3	105 7	3	60 2	12 0
T L	23 7	6	14 9	5	8	1 1	18 0	9	20 4	6	2 4	2 5
T L	24 2	5	5 7	5	6	3 1	13 3	9	24 2	5	10 9	3 2
M D	11 0	4	45 1	4	15	2 3	37 4	2	45 1	4	7 7	3 4
H G	20 0	3	16 9	3	4	0 77	13 9	11	21 5	5	7 6	10 0
H G							7 5	6	25 1	5	17 6	22 0
H G	23 5	2	15 3	4	5	1 6	7 7	5	22 6	5	14 9	9 0
H C	31 6	3	77 0	3	15	3 0	120 3	2	101 1	2	19 2	6 4†
F O	5 6	4	12 9	4	10	0 73	13 7	5	24 6	5	10 9	15 0
F O	21 7	3	45 3	2	5	4 9	5 7	4	15 9	6	10 2	2 1
F O							32 6	11	55 4	3	22 8	4 6

* Difference in 1st two urine sugars divided by units in Column A.

† Column C divided by Column B

‡ Protamine insulin used, unitage equivalent confirmed by difference in fasting blood sugar

Unitage effect of oral insulin

Table III shows a number of estimations of the effect of oral insulin in terms of subcutaneous insulin. This was accomplished in the following manner. From a change in subcutaneous insulin alone, wherever adjacent periods could be obtained for the purpose, the difference was found in the urine sugar at the higher and the lower levels of subcutaneous insulin. This difference in grams was then divided by the number of units by which the two periods differed, for example, with subject T L a change in subcutaneous insulin amounting to 5 units occurred between two adjacent periods, one represented by six days, the other by four. This difference amounted to 3.25 grams. This figure divided by 5 gives the value under Column B, namely 0.64 gram reduction per I U of subcutaneous insulin. This test was immediately followed by a test with oral insulin in which there was a change due to the oral insulin of 10.5 grams. It is therefore reasonable to infer that the dose of oral insulin had a proportionate effect on urine sugar. This is found by dividing 10.5 grams by 0.64, which gives an equivalent of 16 subcutaneous units.

All of the other tests are calculated in the same manner, and it will be observed that the largest effect in terms of subcutaneous insulin was obtained with patient H G, who in a period of six

days on oral insulin showed a reduction in urine sugar amounting to 17.6 grams. In a previous test, from a change in subcutaneous insulin amounting to 4 units there was a reduction in urine of 0.77 grams per unit. Dividing 17.6 grams' reduction by 0.77, we get the effect produced by oral insulin. If this degree of effect could be regularly obtained with diabetic patients, the oral method would be of considerable significance, especially in mild cases.

It was hoped that the fasting blood sugar change might also be made the basis of calculation of unitage effects, but this proved to be disappointing on account of the great variability in the behavior of the fasting blood sugar. In one case, patient H C, who was getting protamine insulin, it was possible to calculate a unitage effect from the change in fasting blood sugar, both from a change of subcutaneous insulin and from the change produced by oral insulin. The figures calculated from urine sugar change and fasting blood sugar change agreed very well.

The range of unitage effect with any single patient was disappointingly large, for example, with subject T L in the later periods of study the unitage effect was only in the neighborhood of 2 to 3 units, whereas at the beginning of the study with him the equivalent effect was, in two periods, 16 and 12 units, respectively. Also with subject

TABLE IV

Evidence of increased combustion of glucose from respiratory metabolism of F O

Date		L. CO ₂ per hour	L. O ₂ per hour	Respira- tory quotient	Calories per hour	Calories from carbo- hydrates	Extra glu- cose burned per hour
1939 February 28	Basal	9.41	9.99	0.94	49.7	40.17	
	After 200 international units oral insulin	11.57	10.25	1.13	53.16	51.73	3.06
March 2	Basal	8.92	10.27	0.87	50.19	28.85	
	After 400 international units oral insulin	11.11	10.51	1.06	53.69	53.04	6.39

F O the unitage effect ranged from 2 to 15 units of subcutaneous insulin

Evidence of increased combustion

On several occasions basal metabolisms were determined on four different patients in the group of twelve listed in Table II. With only one of these, however, was there definite evidence of increased combustion after a dose of oral insulin. Two tests on this patient, F O are given in Table IV. The experiments on February 28 and March 2 were performed in identical manner, except that in the second test twice as much oral insulin was given as in the first. A basal period was obtained early in the morning before breakfast and immediately thereafter the dose of insulin was given. One hour later another respiratory metabolism period was run exactly in the same manner as the basal. The results show in these two tests a nearly proportional effect as between 200 and 400 units of insulin administered orally. For the purpose of such respiratory metabolism experiments the usual carbonate mixture was replaced with a Sørensen phosphate buffering solution, giving a pH of 10. With the carbonate mixture there might be a chance that increased carbon dioxide in the expired air had come from the carbonate mixture. The phosphate buffering solution with this particular patient favored the absorption of insulin quite as well as did the carbonate buffer of the same pH. It is interesting also to note that in this particular patient, F O the phosphate buffering solution raised the carbon dioxide combining power of the blood. Consequently, the actual effect of the oral insulin on combustion was somewhat greater than indicated in the last column. Observe that the principal

effect, notwithstanding increased combining power was upon the CO₂ expired rather than on the O₂ inspired and that respiratory quotients higher than 1.0 were obtained. They are not non protein respiratory quotients, however, and therefore no inference can be drawn regarding fat formation.

It is surmised that, in the several tests on other subjects which showed a good drop in blood sugar but failed to show increased combustion, there actually was some increase but that it was masked by the increased alkalinity of the blood holding back carbon dioxide produced from the extra combustion. Another possibility is that the reduction of blood sugar was caused entirely by increased glycogen formation.

DISCUSSION

The only clinical work with which this investigation can be compared is that of Lasch and Schönbrüner (9). These authors found in previous experiments (10, 11) that acidic dye stuffs such as Congo red and trypan red would completely protect insulin from the destructive effects of pepsin, and that basic dyes such as malachite green and rhodamine would protect it equally against digestive destruction by trypsin. Accordingly for their human experiments they combined these protective agents with dry insulin in a lacquer-covered tablet. Saponin also (Merck and Company) was included as a surface tension lowering agent designed to promote absorption. Since experiments on normal human subjects had proved that none of the adjuvant substances was harmful and that blood sugar lowering could be obtained, the authors proceeded to treat human cases of diabetes in several clinics with such tablets (composition not stated). "Only

any cases were selected, the status of whose metabolism had been established regularly over a sufficiently long fore-period, and with whom a post-experimental observation period appeared to be possible" Results were in hand on more than forty cases at the time the clinical report was written Only eight, however, are represented in the graphs, and no tables are presented

Marked reduction of (fasting?) blood sugar and reduction of urine sugar to the vanishing point or maintenance at low levels by means of oral insulin, are illustrated in all of the eight cases The authors rely upon increases of body weight as proof of the non-interference with digestion and absorption of food by the protecting dye-stuffs This at best is hazardous and in the present instance proves quite inconsistent with the dietary record For example, in one case (Number 5) the "standard diet" is described as "200 grams meat, 50 grams butter, 2 eggs," and contains 50 grams of carbohydrate From these articles alone the patient weighing 63 kgm received only 910 calories, and during the first three days of the chart (without insulin) lost an average of 70 grams of glucose in the urine, or approximately 266 calories Yet during this period she gained in weight! During the next six days with subcutaneous insulin the urine became sugar-free, but the weight did not change!

Another case (Number 3), weighing 61 kgm, on a standard diet "with 200 grams meat, 50 grams fat and 50 grams carbohydrate" during the first ten days of the chart (without insulin) excreted an average of 73.5 grams of glucose but had no acetonuria, and the weight ended where it started The diet described would supply only 845 calories, of which 278 calories were lost in the urine daily

It would seem that the favorable changes in weight with oral insulin must have been caused by constituents of the diet not described How can the authors convince themselves from such data that digestion was not interfered with, or the reader be assured that a great deal of the reduction in urine sugar was not due to partial fasting caused by such interference? No examination of the stools seems to have been made

If trypan red and malachite green protect insulin from digestion in the alimentary tract, why do they not also protect other proteins from di-

gestion, especially as the tablets were given after breakfast and one hour before the mid-day and evening meals and never on a completely empty stomach?

From unpublished experiments in this laboratory with trypan red administered to dogs with insulin and hexylresorcinol, no additional effect has ever been obtained Furthermore, after several doses of trypan red the dogs exhibited red-staining of the sclera of the eyes, skin and toenails, and they did not become entirely free from this coloration for a month or more

The authors' experience with saponin as a promoter of absorption is completely at variance with that of Samek (12), Dingemans and Lacqueur (13), Walton and Bassett (14), and with this laboratory (15) In our experience saponin placed directly in the intestine of an anesthetized dog with insulin interferes with absorption more often than it aids it, as judged by the effect on blood sugar For example, in a recent series of twelve experiments, only one seemed decidedly to enhance absorption, two were doubtful, *i.e.* just on the edge of significance, and nine indicated definite interference with absorption Lasch and Schönbrunner state that their tablets without saponin had no effect at all

Not all of their cases (percentage not given) gave a therapeutically recognizable effect and apparently the authors attribute this failure to an insufficient dosage of saponin to produce absorption Those who did respond favorably gave unmistakable lowering of blood sugar and urine sugar and favorable effects on body weight as well as general clinical condition

The results obtained from the present investigation parallel those of Lasch and Schönbrunner, except for the magnitude of the effects, judging by the few which they disclose In our study, however, we are certain that there was no interference with digestion sufficient to cause loss of nutrients by the stools Since this work started, it has been shown in our laboratory² that hexylresorcinol does interfere with the proteolytic action of pepsin, trypsin and erepsin, but the effect *in vivo* must be quite fleeting so far as digestion of food proteins is concerned We have pur-

² These results were obtained by Mr. R. L. Driver and will be published later

posely limited the use of adjuvant substances to the point where the over all digestion in twenty-four hours was demonstrably normal

SUMMARY

1 Twenty cases of human diabetes of varying degrees of severity have been employed in this study of the effects of hexylresorcinol and a buffering mixture of salts on the absorption of insulin from the alimentary tract

2. The "standard dose" was 100 I U pure insulin in a solution containing 0.125 per cent hexylresorcinol and alkaline salts necessary to give a pH of approximately 10 to 15

3 As many as eight such standard doses can be given in one day without interference with total digestion, the last dose being administered not later than 11 p.m. and the twenty-four-hour urine period closing at 7 a.m. Diets were kept constant for control and experimental periods.

4 Detailed results on urine sugar for twenty-four hours, and blood sugar change early in the morning from the first dose of oral insulin, are presented for the twelve cases who reacted most favorably. These show weighted average effects on urine sugar varying from 4.7 grams to 17.7 grams per twenty-four hours below the average excretion in control periods. The blood sugar effect from the single dose, again as a weighted average, varied from nothing (Case H. C.) to 60 mgm per cent in forty five minutes (F. O.). The total insulin per twenty four hours varied from 200 or 300 I U to 800 I U. The effects on urine sugar were not proportional to dosage, often being as great with 200 to 300 I U as with 600 to 800 I U. Greater effects were obtained when urine sugar and fasting blood sugar were high than when they were low.

5 The equivalent unitage effects of oral insulin in terms of subcutaneous (regular) insulin varied from 2.1 I U to 22 I U the average of five cases in which this assay was possible being for all doses of oral insulin just under 10 I U of subcutaneous insulin.

6. In a single case it was possible to make out an effect nearly proportional to a dose of oral insulin, and in this case also to demonstrate an effect on respiratory metabolism which could be calculated in terms of glucose oxidized.

7 These results are only mildly encouraging to the hope of adequate control of diabetes mellitus with oral insulin

CASE ABSTRACTS

T. L. A 23-year-old white male found to have diabetes mellitus in 1930. Diet at home previous to admission calories, 2083 protein, 78 fat, 101 carbohydrate, 201 with insulin a.c. 30-20-28. Admitted for metabolic study December 26, 1937. Placed on diet as follows: calories 2075, protein 80 fat, 100 carbohydrate, 200. Continued on this diet through the administration of oral insulin. Subcutaneous insulin before oral started, 25-5-10. On February 6 diet was increased to calories 2558 protein, 80, fat, 123 carbohydrate, 265. Continued on this diet as oral was again started March 2 1938. Subcutaneous insulin before oral, 14-12-12-10. Discharged and returned May 27 1938 to re-enter diabetic study. Diet as follows: calories, 2558 protein, 80 fat, 123, carbohydrate, 265. Continued on this diet through oral administration. Subcutaneous insulin before oral, 30-0-12-6.

M. D. A 20-year-old white male. Known diabetic since 1931. Insulin at home just previous to admission December 27 1937 not recorded. Last record of insulin taken at home that is stated in history was October 30 1936, when patient was discharged from hospital on 3500 calories with protamine insulin 30 units and regular insulin 25 units a.c. breakfast. Admitted for diabetic study December 27 1937. Placed on following diet: calories, 1850 protein, 65, fat, 90 carbohydrate, 182. Continued on this diet, although there were several slight changes due to addition of orange juice for insulin reaction. Subcutaneous insulin before oral started, 20-5-5-10.

H. G. This 42 year-old white male had first symptoms of diabetes in 1928 at the age of 32 years. Diet at home previous to admission: calories, 1646, protein, 70 fat, 80 carbohydrate, 150 with insulin a.c. 35-10-35. Admitted for metabolic study February 16, 1938. Placed on following diet: calories, 2150 protein, 76 fat, 105 carbohydrate, 210. Continued on this diet until administration of oral insulin. Subcutaneous insulin before oral stated, 12-10-12-10. Discharged April 3 1938 to return in one week or ten days. Re-admitted for diabetic study April 19 1938. Placed on same diet as in previous study: calories, 2150 protein, 76, fat, 105 carbohydrate, 210. Continued on this diet through oral administration. Subcutaneous insulin before oral started, 15-10-12-10.

J. H. A 21 year-old white male who was first found to be a diabetic at the age of 10 years. Diet at home previous to admission: calories, 2060 protein, 80, fat, 100 carbohydrate, 196 with insulin a.c. 40-0-30. Admitted for diabetic study February 16, 1938. Placed on following diet: calories, 2050, protein, 75 fat 100, carbohydrate, 198. Continued on this diet with the administration of oral insulin. Subcutaneous insulin before oral started, 14-12-10-6.

H. C. A 17 year-old white male first showed symp-

toms of diabetes in 1936 Diet at home previous to admission calories, 2570, protein, 107, fat, 116, carbohydrate, 257, with protamine insulin 40 units a.c. breakfast and regular insulin 20 units a.c. supper Admitted for diabetic study April 19, 1938 Placed on diet as follows calories, 2570, protein, 107, fat, 125, carbohydrate, 236 Continued on this diet through the administration of oral insulin Subcutaneous insulin before oral started, protamine 15 units, regular 10 units

L P A 23-year-old white male. First knowledge of diabetes in 1936 Diet at home previous to admission calories, 2450, protein, 133, fat, 92, carbohydrate, 257, with insulin s.c., protamine insulin 50 units, regular insulin 10 units in the a.m. Admitted for metabolic study June 4, 1938 Placed on the following diet calories, 2460, protein, 90, fat, 120, carbohydrate, 238 Continued on this diet through the administration of oral insulin Subcutaneous insulin before oral started, 10-0-5-0

T V A 16-year-old Italian boy first showed symptoms of diabetes at the age of 12 Diet at home calories, 2125, protein, 82, fat, 122, carbohydrate, 184, with protamine insulin 35 units, and regular insulin 15-0-20 Admitted for diabetic study April 19, 1938 Placed on diet calories, 2020, protein, 82, fat, 100, carbohydrate, 184 Continued on this diet with administration of oral insulin Subcutaneous insulin before oral started, protamine 15 units, regular 10 units, later changed to protamine 15 units, regular 25 units

F O A 34-year-old white male with diabetes of one year's duration. Diet at home (a gap of eight months in history) not recorded Admitted for diabetic study January 4, 1939 Placed on the following diet calories, 1907, protein, 78, fat, 94, carbohydrate, 174 Final constant diet calories, 1980, protein, 78, fat, 107, carbohydrate, 177 Subcutaneous insulin before oral started, 10-0-0-0

P F A 56-year-old white male who has been a known diabetic since 1930-1931 Diet at home before admission 1800 calories Insulin taken at home 25-0-15 Admitted for special study December 27, 1938 Placed on the following diet calories, 1760, protein, 68, fat, 100, carbohydrate, 147 Final constant diet before oral administration calories, 1764, protein, 69, fat, 100, carbohydrate, 147 Subcutaneous insulin before oral, 30-0-15-0

P J A 57-year-old white woman, a known diabetic for ten years Diet at home 1600 calories Protamine insulin daily at home, 25 units Admitted for study June 6, 1939 Placed on the following diet calories, 1646, protein, 62, fat, 80, carbohydrate, 158 Continued on this diet through diabetic study Subcutaneous insulin before oral started, 10-0-0

E W A 32-year-old Negress, a known diabetic for nine years Protamine insulin (taken at home) 30 units, regular insulin 10 units, a.c. breakfast daily Admitted for diabetic study June 6, 1939 Placed on following diet calories, 1230, protein, 60, fat, 70, carbohydrate, 135 Continued on this diet through study Subcutaneous insulin before oral started, 25-10-10

W V A 47-year-old minister who apparently had had diabetes for about nine years Diabetes first discovered

three years ago Regular insulin, 25 units daily, at home (History does not state when this was taken) Admitted for study June 23, 1939 Placed on following diet calories, 2120, protein, 70, fat, 105, carbohydrate, 210 Continued on this diet through diabetic study Subcutaneous insulin before oral started, 25-0-0

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BRONCHIAL CALIBRE CHANGES IN BRONCHIECTASIS¹

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The modern tendency to plan for lobectomy upon a diagnosis of bronchiectasis (1 to 9) makes it mandatory that we possess as much knowledge as possible about the condition.

Since the disease is one of the bronchi and bronchioles these structures become of marked interest to us. It is known that the calibre of normal bronchi increases at the end of inspiration and diminishes at the end of expiration (10) and at the end of coughing. That there is to some extent nervous control of these calibre changes has also been demonstrated (11). Stimulation of the vagus constricts the bronchi and bronchioles and stimulation of the sympathetic nerves dilates them (12, 13). By vagal irritation in the bronchial mucosa the cough reflex is initiated.

Pathological investigations have shown fairly constant changes in established bronchiectasis. There is lymphocytic infiltration of all the layers of the bronchial wall. There are ulcerative changes in the mucosa and destruction of the flexible elements, namely the muscle, cartilage and elastic fibers in the bronchial wall. There is also a transformation of the normal columnar ciliated epithelium of the mucosa to that of stratified squamous epithelium (14). As in tissues elsewhere in the body the destroyed elastic elements are replaced by fibrous tissue (15). The mucosal changes cause loss of the cough reflex. The pathological changes in the bronchial walls produce loss of tone and stagnation of infected secretions, resulting in the gross picture of dilated or tubular bronchi and saccular bronchioles filled with purulent material. The secretions cannot be coughed up until they overflow onto normal mucosa where the cough reflex can be initiated. Consideration of the above pathological and physiological changes has led to the conclusion that they are irreversible and that only removal of the involved lobes would affect a cure (2, 15).

The introduction of iodized oil (Ipiodol, La

foy) by Forestier and Sicard (17) and the simple method of instilling it into the bronchial tree originated by Sipper (18) made it possible to outline the air passages with ease. Taking advantage of this method Heimbecker (10) in 1927 studied the calibre changes in the bronchi of humans and animals during normal respiration. He found as mentioned above that the bronchi and bronchioles widened at the end of inspiration and narrowed at the end of expiration. These changes occurred even when the lungs were removed from the influence of the nervous system. He explained the narrowing and widening on a passive basis as resultants of linear and radial tractions, the character and relative degrees of which are determined by the manner of enlargement of the thoracic cavity during respiration.

It occurred to the author that the bronchi of individuals with bronchiectasis could be similarly investigated. One normal individual and four patients with established bronchiectasis thus studied are presented in this report.

METHOD

After instillation of the iodized oil by the Singer method, the bronchial tree in the affected lobe was examined under the fluoroscope for calibre changes on deep inspiration and deep expiration. For permanent records, x-ray films were taken while the breath was being held at these extremes of respiration. All radiographs were taken at a constant distance, three feet and all exposures made very quickly to avoid the effects of motion in the films. Suitable markers were placed on the plates to identify the phases of respiration with which they were associated.

RESULTS

For the purposes of comparison with the findings in the patients with bronchiectasis normal individuals were first studied in the above manner. One such normal subject is presented (Figure 1). It can be seen in his bronchograms that the bronchi and bronchioles are largest at deep inspiration and smallest at deep expiration. In

¹This work was aided by a grant from the Comly Fund for Research of the Ohio State University.

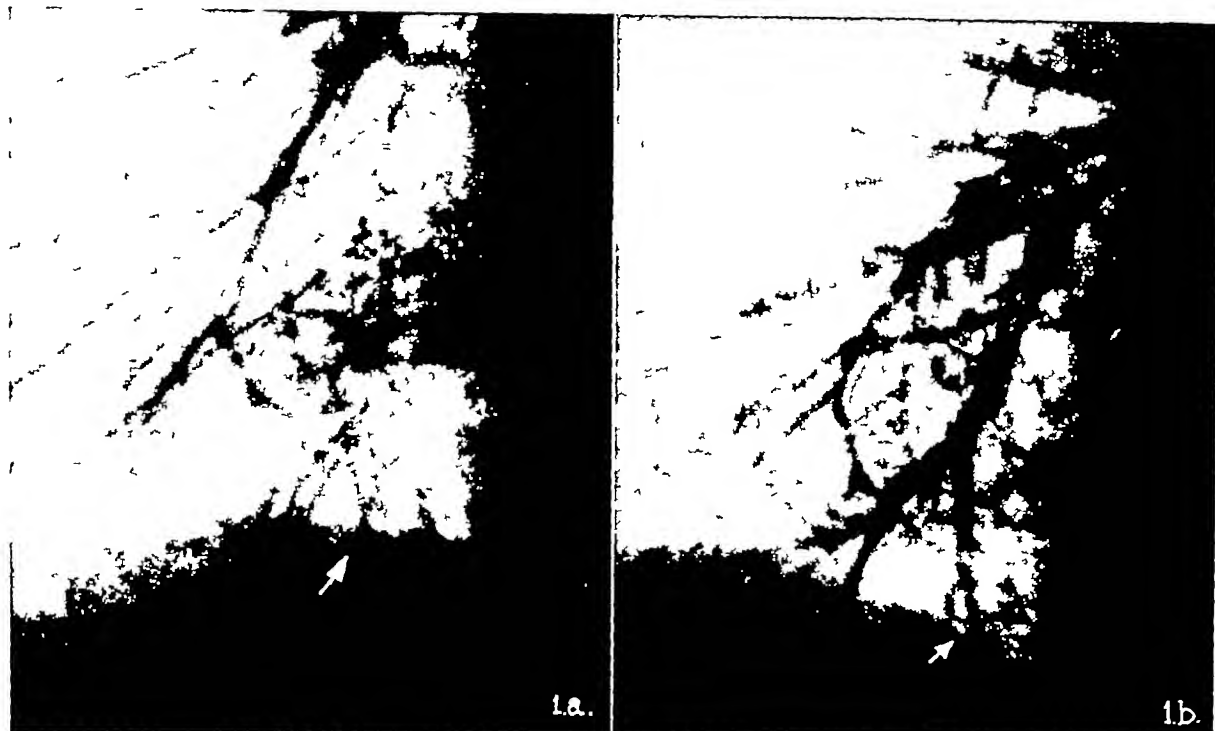


FIG 1 BRONCHOGRAMS OF REGION OF RIGHT LOWER LOBE IN A NORMAL INDIVIDUAL

No 1-a taken at the end of deep inspiration Radiograph No 1-b taken at the end of deep expiration All the bronchi and bronchioles in 1-a are decidedly larger Arrow points to the same bronchus in both Note that the indicated bronchus at expiration is about one-third the diameter of that on inspiration

order for this to occur, the elastic elements in the bronchial walls must be intact

Cases with 'wet' bronchiectasis were then studied in a similar manner to determine the response of the diseased bronchi and bronchioles Short histories with the results of the investigation in two such cases follow

E G This 19-year-old white boy was first seen in the Thoracic Surgery Clinic at the University Hospital in October, 1939 Fifteen years prior he had had 'pneumonia' and since then he had had a chronic cough productive of large amounts of foul, purulent sputum He had consumed a large amount of cough syrup during these years, without relief Upon examination, there were heard numerous coarse rales in the left lower chest Clubbing of the fingers and toes was present The breath was foul The sputum was negative for acid-fast organisms Bronchograms after lipiodolization showed tubular and saccular bronchiectasis in the region of the left lower lobe When studied according to the method of Heinbecker, the affected bronchi did not change in calibre at the inspiratory or expiratory extremes of respiration (Figure 2) The normal bronchi above the affected region, however, did respond in the physiological manner This patient is being prepared for lobectomy

by repeated postural drainage and therapeutic lipiodolizations

R A This 19-year-old white girl was first seen in the Thoracic Surgery Clinic at the University Hospital in November, 1939 She had had a chronic cough, productive of large amounts of purulent sputum since childhood Recently she had begun to have occasional attacks of hemoptysis and, therefore, had been hospitalized in a tuberculosis sanatorium Repeated examinations of the sputum were negative for acid-fast bacilli Upon examination in the clinic there were found coarse moist rales over both lower lobes, poor motion of the chest upon respiration, clubbing of the fingers and toes, and signs of weight loss Fluoroscopic and x-ray examination, after injection of the bronchial tree with lipiodol by the Singer method, showed tubular and saccular bronchiectasis in both lower lobes There was no change in the calibre of the bronchi or bronchioles in either lower lobe upon deep inspiration or deep expiration The bronchogram of the right lower lobe is shown in Figure 3 Since the patient is sensitive to iodine she is being prepared for bilateral lower lobectomy by postural drainage If postural drainage does not rid her of the infected secretions, x-ray therapy will be used to 'dry' her bronchiectasis

From the above it became apparent that these diseased bronchi are incapable of the normal calibre changes Some still cling to the belief that

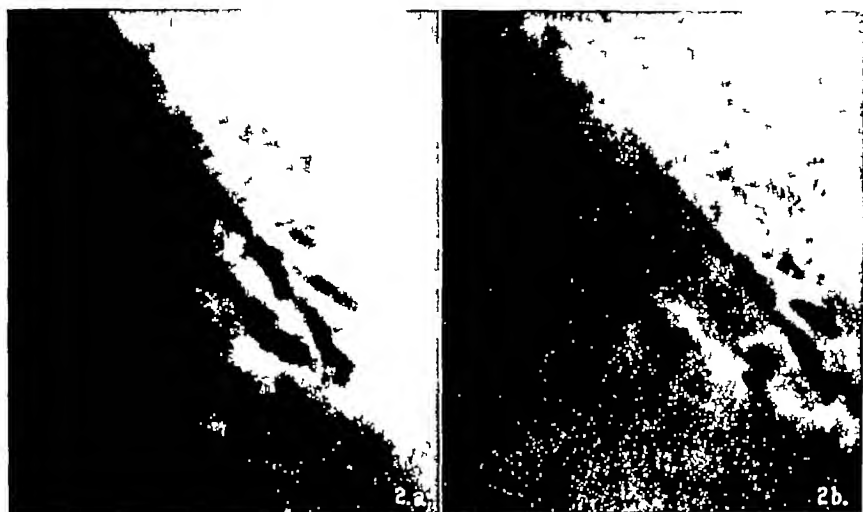


FIG. 2. BRONCHOGRAM OF REGION OF THE LEFT LOWER LOBE IN A CASE OF ESTABLISHED "WET" TUBULAR AND SACULAR BRONCHIECTASIS.

No. 2 a taken at the end of deep inspiration and No. 2 b at the end of deep expiration. Note absence of change in calibre of bronchi and bronchioles at the two extremes of respiration.

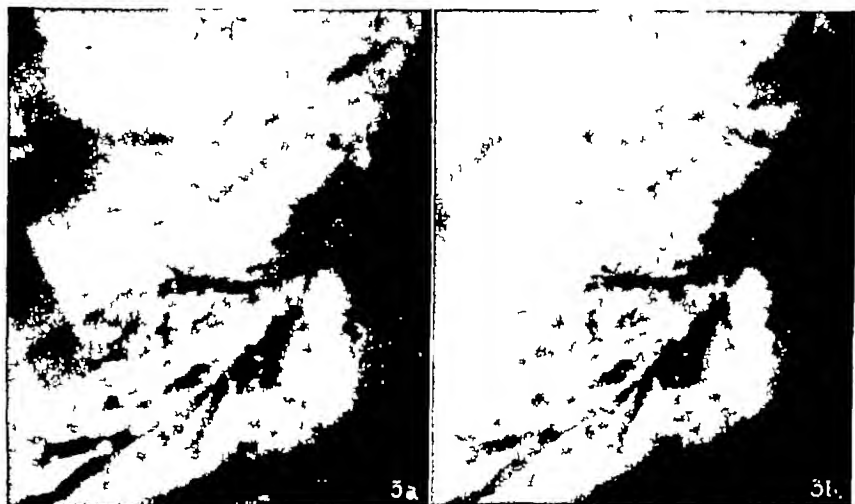


FIG. 3. BRONCHOGRAMS OF REGION OF THE RIGHT LOWER LOBE IN A CASE OF BILATERAL "WET" TUBULAR AND SACULAR BRONCHIECTASIS.

No. 3-a taken at deep inspiration and No. 3 b at deep expiration. There is no change in the calibre of the affected bronchi at these phases of respiration.

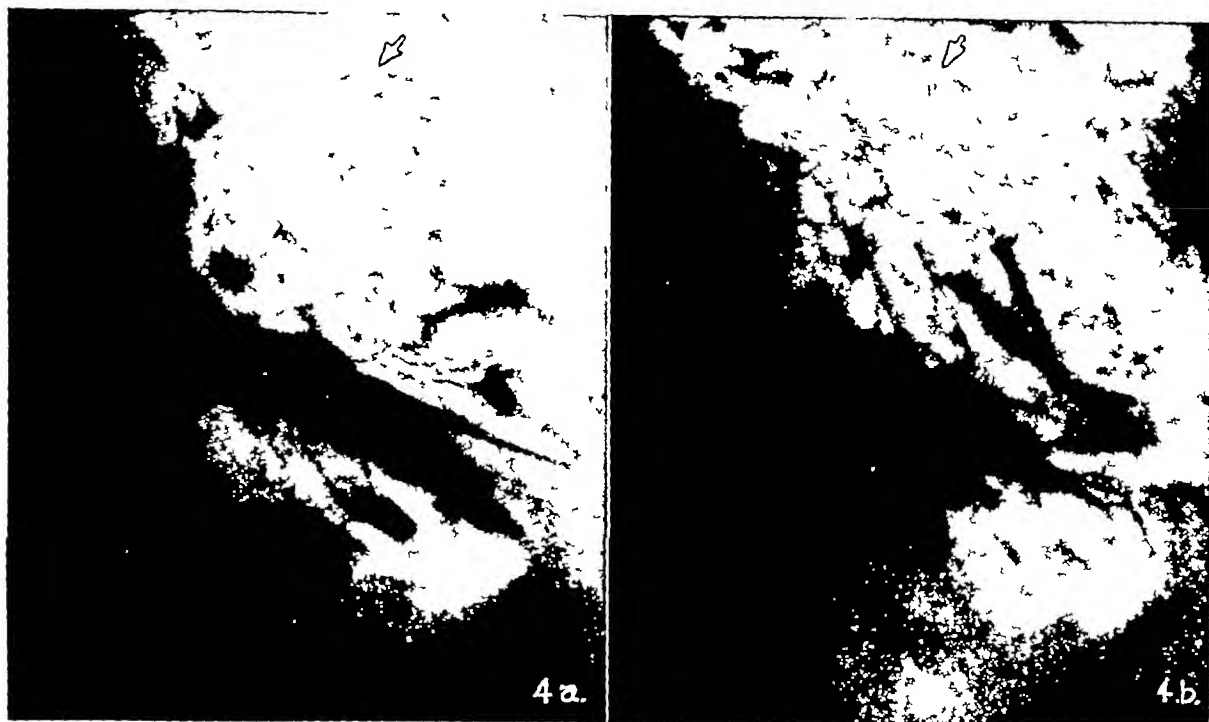


FIG 4 BRONCHOGRAM OF LEFT LOWER LOBE IN A CASE OF "DRY" BRONCHIECTASIS

No 4-a taken at deep inspiration and No 4-b at deep expiration. There is no change in calibre of the affected bronchi at the extremes of respiration, but the normal bronchi in the upper part of the lobe do show the expected changes (arrows)

by making cases of bronchiectasis symptom-free, cure is effected. To determine whether such is the case, individuals made "dry" by conservative measures were studied in the manner described for those with "wet" bronchiectasis. Two of these, with results are presented.

J C This 26-year-old white man was seen on the Research Surgical Service at the University Hospital in May, 1939. He had a history of cough productive of large amounts of purulent sputum of twenty-two years' duration. He could not remember a predisposing illness, but had been bothered with chronic sinusitis all his life. Examination revealed coarse, moist rales in the right lower lobe. The sputum contained no acid-fast bacilli. Bronchograms showed tubular and saccular bronchiectasis in the lower part of the left lower lobe. With daily postural drainage and bimonthly therapeutic use of lipiodol he became "dry" in three months. Five months after becoming dry he was studied according to the method of Hembecker. Although he was symptom-free, there was no morphological improvement and the dilated bronchi and bronchioles did not change in calibre at the extremes of respiration. However, the unaffected bronchi in the upper part of the lobe responded in the normal manner (Figure 4). This patient is to have a left lower lobectomy when his sinusitis is corrected.

H M This 24-year-old white boy was made "dry" by treatment and had a right lower lobectomy for bronchiectasis in 1938. On subsequent examination after instillation of lipiodol, it was found that the bronchi of the right middle lobe, which had expanded to fill the residual space, were also ectatic. He was, nevertheless, symptom-free. Evidently because of inadequate visualization of the entire bronchial tree, before operation, this involvement was not recognized. When studied by the Hembecker method there was no change in the calibre of the affected bronchi or bronchioles (Figure 5). He is at present being treated by means of lipiodol every two months and precautions are taken to prevent upper respiratory infection. He will eventually need to have a lobectomy of the right middle lobe.

Apparently, there is no improvement in the morphological appearance or in the physiological reaction of the affected bronchi when the bronchiectasis is made "dry" and the patient symptom-free.

DISCUSSION

The conclusion arrived at by Biermer (14) and Warner (16) from their respective pathological and clinical observations was that the changes exhibited in bronchiectasis are irreversible when managed by conservative measures. Their con-

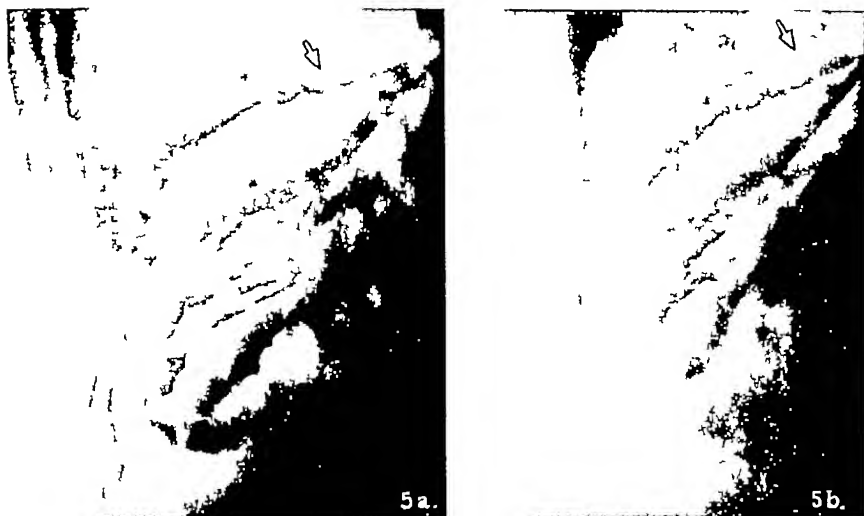


FIG 5 BRONCHOGRAM OF RIGHT LOWER LOBE IN A CASE OF "DRY" BRONCHIECTASIS IN WHOM THE RIGHT LOWER LOBE HAS BEEN REMOVED

No. 5 a taken at deep inspiration and 5 b at deep expiration. No change in the calibre of the affected bronchi can be seen. Note however the change in calibre in the normal bronchus (arrow)

tention is further borne out by these studies. The persistent tubular and saccular ectasia of the bronchi as well as their inability to respond normally at the extremus of respiration in the cases of bronchiectasis made dry by treatment is clear evidence that the pathology remains unchanged when symptomatic improvement is obtained.

It is believed that no matter what initiates the series of events leading to the final picture of bronchiectasis there must be an infection of the bronchial walls a bronchitis preceding the final destructive process. It may be that there is a phase before destruction of the flexible tissues becomes marked where the condition is still reversible. Ochsner (19) reported four cases in which he obtained morphological as well as symptomatic cure and Berck and Harris (20) reported some cases in which they not only obtained symptomatic improvement but shrinkage of the dilated bronchi. The former used postural drainage and repeated treatment with lipiodol and the latter used Roentgen irradiation as the method of management.

It has not been the good fortune of the author to obtain such 'early' cases of bronchiectasis for study. It is possible that the ectatic bronchi and bronchioles in such cases might become narrower in calibre upon deep expiration and wider upon deep inspiration. By this method of study it would be possible to select certain cases for conservative management instead of preparing them for lobectomy as is now customary in well established cases.

CONCLUSIONS

- 1 The calibre changes in the bronchi of normal individuals and in cases of wet and dry bronchiectasis were studied by the method of Heinbecker.

- 2 Whereas the unaffected bronchi were found to be dilated upon inspiration and constricted upon expiration the affected bronchi in the wet cases did not change in calibre. Particularly notable was the lack of constriction on deep expiration.

- 3 The affected bronchi and bronchioles in the case of bronchiectasis made dry retained their

ectatic state and likewise did not change in calibre at the extremes of respiration

4 These studies lend evidence to the opinion that well-established bronchiectasis is irreversible and that surgery is indicated for cure

5 It is suggested that by this method early cases, in which the marked destruction of the flexible elements of the bronchial walls has not yet occurred, might be recognized by the fact that the bronchi remain capable of changing in calibre at the extremes of respiration. Such cases could be treated conservatively instead of surgically

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STUDIES IN RHEUMATIC DISEASE. V THE AGE AT ONSET OF PRIMARY RHEUMATIC ATTACK

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The literature dealing with the age at onset of rheumatic disease is confusing to the reader. There is not only lack of agreement among the various authors with respect to the age at which the disease is most likely to appear, but also the evidence presented in support of the varying conclusions is not convincing (1 to 23). The published data on this subject are open to criticism because in most instances they have one or more of four main defects:

1. The data are restricted to a consideration of only one of the various manifestations of the disease. Rheumatic disease manifests itself in a variety of forms and the age selection is by no means similar for all syndromes so that data which are restricted to a consideration of only one manifestation do not give a true picture of the age selection of the disease as a whole.

2. The data are obtained from the analysis of groups of cases which are, in themselves, age limited. For this reason the age of greatest susceptibility is found by the pediatric clinic to be in childhood while clinics with an adult clientele report the maximum incidence to be in adult life.

3. The data are based upon the occurrence of acute attacks of the disease without reference to whether the attack represents an onset or a recurrence. Rheumatic disease is generally regarded as a chronic recurrent condition. The age selection of the disease should, therefore, be determined on the basis of the age at which the disease had its onset, and thus, with our present diagnostic standards, is in most instances approximated by the age at which it first became clinically manifest.

4. The analysis deals with the age at onset of the disease without reference to the universe from which the cases are drawn. In other words, no consideration is given to the age distribution of the population at risk from which the cases arise.

It is not a simple matter to obtain data on rheumatic disease free from all of the above defects. At the present time no satisfactory data are available relative to the occurrence of the disease in the general population and because this information is lacking the age selection of the disease must be studied in particular groups of individuals. It is difficult to obtain such a group with any certainty that it is not biased in some form. The question of the age selection of the disease is, however, of sufficient importance to merit further investigation. The purpose of this paper is to present and discuss the results of a study of the age susceptibility to rheumatic disease based on certain information collected in the Cardiac Clinic of the Harriet Lane Home.

MATERIAL AND METHODS

The data upon which this report is based were abstracted from the medical histories of 96 consecutive admissions of white children to the Cardiac Clinic of the Harriet Lane Home because they were suffering from some rheumatic manifestation. These histories include a careful epidemiological study of the immediate families of these children and the families of their parents so that information is available regarding the occurrence of rheumatic disease in the patients and their siblings, parents, grandparents, uncles and aunts. The children who were admitted to the clinic suffering from the disease, and who were responsible for the inclusion of their respective families in the study are denominated "index cases" to distinguish them from their relatives who entered the study because of their relationship to these cases. Each index case, therefore, must, by definition, have had an attack of rheumatic disease during childhood. Such is not the case with their relatives who may or may not have had rheumatic disease.

In this study as in the four preceding ones (24 to 27) an attack of rheumatic disease was defined as either Chorea, Rheumatic Polyarthritides (Rheumatic Fever) or Rheumatic Carditis, or some combination of two or more of these three syndromes.

The following information is available each of the index cases and their near

informant (usually a parent of the index case) may not only fail to remember illnesses among his siblings but may also forget his own, particularly those which occurred in early life. This can have two effects (1) Cases which have their onset early in life may not be reported, and (2) a recurrence may be called a first manifestation, which means that it is recorded as occurring at a later age than the actual onset of the disease. The tendency of this error is, therefore, to decrease the number of cases with onset early in life and to increase the number of cases with onset later in life.

The other main error is due to the fact that following the dispersal of the family group by marriage, *etc*, other members of the family may have developed the disease unknown to the informant. The effect of this error is to reduce the number of cases with onset in adult life. These two errors are inherent in this method of collecting information, their tendency, however, would be to counterbalance each other. In this study, in so far as possible, such errors have been reduced to a minimum. The histories were carefully taken and checked, and about 70 per cent have been verified by hospital records.

The age at onset of rheumatic disease

The age at onset of rheumatic disease has been defined, for purposes of this study, as the age at which the patient suffered his first attack with one of the three manifestations (Chorea, Polyarthrititis, or Carditis). Among the 971 members of this generation upon whom histories were obtained, 132 individuals had definite histories of having some form of the disease, and in 115 of these the age at onset was definitely stated. The remaining 17 were unable to give the age at onset, although the history of the disease was quite definite. This failure to supply complete information was due partly to defective memory on the part of the informant and partly to the fact that in some instances (10 of 17) the disease was discovered as chronic rheumatic carditis without having shown clinical evidence of the acute disease at any time. The 115 in whom the age at onset was known are shown in Table IV by age and type of first acute manifestation. The age at onset by single years of these 115 cases is also shown graphically in Figure 1.

TABLE IV

Age at onset of cases of rheumatic disease occurring among the parents, uncles and aunts (one generation) of index cases

Age	Total	Type of first manifestation*			
		Chorea	Rheumatic polyarthrititis	Rheumatic carditis	Two or more syndromes†
4 years	4	1	3		
5-9	20	6	11	1	2
10-14	27	10	16		1
15-19	12	1	9	2	
20-24	12		10	1	1
25-29	16		13	2	1
30-34	15		14	1	
35-44	9		5	3	1
Unknown	17	1	6	10	
Total	132	19	87	20	6

* Cases are listed here according to the manifestation on first attack of the disease. The relationship between the type of manifestation on first attack and subsequent manifestations was

Manifestation on first attack		Showing other types of manifestations on subsequent attacks
Chorea	18 cases	9 cases
Rheumatic polyarthrititis	81 cases	22 cases
Rheumatic carditis	10 cases	none

† The onsets recorded here were

Two cases of Chorea with Rheumatic Polyarthrititis at 5 and 8 years of age
One case of Chorea with Rheumatic Carditis at 10 years of age
Three cases of Rheumatic Polyarthrititis with Rheumatic Carditis at 24, 28 and 40 years of age

A study of this table and figure indicates that in this generation individuals who developed the disease had their first manifestations at all ages up to 45 years. The onsets occurred most frequently between the ages of 5 and 15 and 25 and 35 years and there were fewer cases who had their first clinical manifestations between 15 and 25 years.

The syndrome by which the disease was first manifested varied with the age at onset. In 20 of the 51 cases whose onset was under 15 years of age the first manifestation of the disease was an attack of chorea, either alone or accompanied by rheumatic polyarthrititis or rheumatic carditis, and in only one instance at this age was the first manifestation an attack of rheumatic carditis alone. The cases with onset after their fifteenth year showed a reversal of this picture, only 1 of the 64 cases manifesting itself first as chorea, while 9 appeared first as rheumatic carditis alone. The number of cases whose onset was indicated

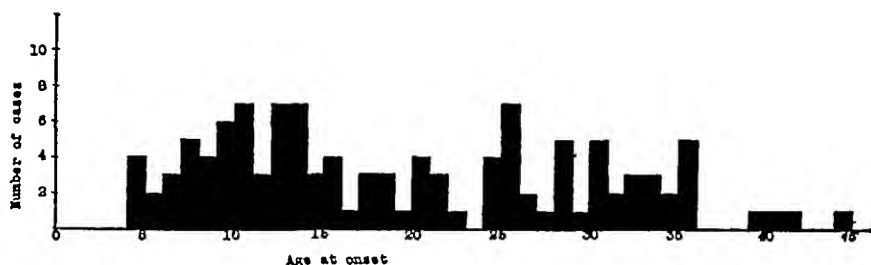


FIG. 1 AGE AT ONSET OF PERSONS WITH RHEUMATIC DISEASE AMONG PARENTS UNCLAS AND AUNTS OF INDEX CASES

by an attack of rheumatic polyarthritis appeared to be uninfluenced by age.

The age susceptibility of rheumatic disease

The foregoing analysis has been confined to a consideration of the age at onset of those persons among the members of this generation who have already developed the disease and no consideration has been taken of the fact that all cases of rheumatic disease may not yet have manifested themselves, nor of the number of persons at risk at the various ages from which the cases arose. In order to compare the risk of developing the disease at different ages it is necessary to consider not only the actual cases which occur, but also the number of persons and the time period during which they were at risk of contracting the disease during each age period. In other words, the cases must be considered in relation to the universe in which they occurred.

The unit which has been used to measure the population at risk during each age period is "person-years at risk" and this unit is defined as 1 person at risk of developing the disease for 1 year. To obtain this population each person was considered at risk from their third birthday and to have remained at risk for a full year at each year of life down to the age at which the first of three possible events occurred (1) death, (2) onset of rheumatic disease, and (3) termination of observation. At this later age the individual was considered to be at risk for one half year because the mean time of the occurrence of the event would fall midway between birthdays.

The population at risk, the cases having their

TABLE V*

Showing the population at risk the cases of rheumatic disease with onsets and the annual attack rates by age groups among one generation (parents uncles and aunts) of the families of 96 rheumatic index cases

Age	Population at risk (person-years)	Cases of rheumatic disease (onsets)	Annual rate per 1,000
3-4	1,890.0	4	2.1
5-9	4,607.5	20	4.3
10-14	4,381.5	27	6.2
15-19	4,209.5	12	2.9
20-24	3,991.0	12	3.0
25-29	3,627.0	16	4.4
30-34	3,129.5	15	4.8
35-44	3,906.5	9	2.3
45+	1,479.5		
Total over 2 years	31,226.0	115	3.7

* The 17 cases of rheumatic disease in which the age at onset was unknown have been omitted from this table. The data, therefore refer to the experience of 954 individuals.

onset and the annual attack rates are shown, by age groups, in Table V

It will be noted that for this generation of rheumatic families the mean rate of incidence (onsets) at all ages is 3.7 per 1,000 per year. The risk of manifesting the disease for the first time rose above this mean during two age periods, from 5 to 14 and from 25 to 34 years of age. The incidence was high after the fifth year of life and reached its maximum from 10 to 14 years of age to be followed by a significant decline during the next decade of life. Between 25 and 34 years of age the incidence was again maturing the high rates noted in after the incidence declined corded as having their onset. In interpreting this latter

membered that the tabulations with respect to the older ages are based upon only a sample of the final experience of this generation at these ages

SUMMARY AND CONCLUSION

Various defects in published data relative to the age selection of rheumatic disease are discussed, and the results of a study on this subject, in which every effort was made to eliminate these errors, are presented

The group for study was a generation of the families of 96 consecutive admissions because of rheumatic disease to the Cardiac Clinic of the Harriet Lane Home, and consisted of parents, uncles and aunts of these admissions (index cases).

The accuracy of the data and various possible errors in the histories are recognized and discussed

The age at onset of 115 cases of rheumatic disease occurring in the generation was found to fall most frequently between the ages of 5 to 14 and 25 to 34 years

The relative risk of developing the disease was determined by dividing the number of onsets at each age by the person-years at risk for the corresponding age to obtain an annual incidence rate. This risk was found to be greatest between the ages of 5 to 14 and 25 to 34 years

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ANTIBODY FORMATION IN CASES OF LOBAR PNEUMONIA TREATED WITH SULFATHIAZOLE

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Early in 1940 we published a report (1) on antibody formation in cases of lobar pneumonia treated with sulfapyridine. In nineteen treated cases of pneumococcus pneumonia repeatedly studied during the acute phase of the disease and during convalescence, an excess of type specific antibody was only demonstrated in the serum of four. In the past it has often been shown that spontaneous recovery from lobar pneumonia is associated, in the great majority of instances, with the appearance of an excess of antibody in the patient's serum and this phenomenon has been generally regarded as an essential part of the mechanism of spontaneously occurring crisis. Our failure to demonstrate antibodies in fifteen out of nineteen cases suggested to us that sulfapyridine had supplanted, at least to some degree, this portion of the immune process. We did not believe that antibody production ceased to exist in treated cases but rather that it proceeded at a lower rate, probably because the stimulus to antibody formation was lessened as a result of the action of the drug on the invading organism. Our technique for the demonstration of excess antibody throughout the series studied was the precipitin reaction with type specific polysaccharide.

While this paper was in press, two similar studies were published. Wood and Long (2) reported the appearance of mouse protective antibodies in the serum of ten out of eleven treated patients recovering from lobar pneumonia but pointed out that in seven of these cases antibody appeared after the time of "essential recovery." Subsequently, Finland and his associates (3) reported a large series of cases of pneumococcus pneumonia in which mouse protective antibodies and agglutinins were sought. They concluded as a result of these studies that "the antibody response of patients with pneumococcus pneumonia treated with sulfapyridine as far as could be determined, was comparable in every respect to that

resulting from spontaneous recovery." It will be observed that these results were not in accord with our findings. None of these investigators, however, used the same technique as we did—to wit, the precipitin reaction. It is our belief that the precipitin reaction, as Heidelberger and Kendall (4) have pointed out, is probably less sensitive than the other methods, but that it is more clear-cut and more specific. Unfortunately, at the time our paper was written, we had no control series of our own in which untreated patients were studied by the same technique, although such was present in the literature (5). As will be observed, the results which we obtained in patients treated with sulfathiazole as opposed to sulfapyridine, using precisely the same technique, were almost diametrically different and therefore constitute a very satisfactory control of the method.

Before sulfathiazole was introduced, eleven additional sulfapyridine treated cases were studied in the same manner as those reported in the original paper. Including one doubtful reaction only four of these showed precipitins during convalescence. Adding these to the original series, we have then a total of thirty cases of pneumococcus pneumonia treated with sulfapyridine of these twenty-two, or nearly seventy five per cent, recovered from the disease without the appearance of an excess of antibody in the blood serum as demonstrated by the precipitin reaction.

The chief purpose of the present communication is to describe the results which we obtained when an exactly similar study was made of patients treated with sulfathiazole.

MATERIALS AND METHODS

These were precisely the same as those employed in the preceding paper (1), and will not be described in detail. The precipitin reaction

with type-specific polysaccharide was employed throughout

RESULTS

The accompanying table gives our data on twenty-one cases of lobar pneumonia treated with sulfathiazole which were investigated serologically for the appearance of type-specific antibodies by means of the precipitin reaction with specific

polysaccharide. Altogether ten different types of pneumococcus pneumonia are represented. It will be observed that circulating antibody was detected by this means in sixteen out of the twenty-one cases. Of the five which failed to show antibody, two were type i, and three types iv, v, and xiv, respectively.

The time of appearance of the antibody is of considerable interest. Of thirteen cases in which

TABLE I

Type specific antibodies investigated in twenty-one cases of sulfathiazole-treated pneumonia

Case number	Sex	Age	Duration of disease before treatment	Type	Lobe	White blood cells	Temperature before treatment	Date treatment started	Date temperature normal	*Precipitin tests	Total dose sulfathiazole	Remarks
1	M	57	1 day	ii	LLL	35 200 P 92%	104.8	December 4	December 5	December 6 00 December 8 00 December 9 00 December 11 00 December 13 ++ December 15 ++ December 16 ++ December 18 0+ December 20 ++ December 23 ++	grams 26	
2	M	21	1 day	xxi	RLL	36 800 P 91%	106.4	January 24	January 25	January 26 00 January 29 00 January 30 00 January 31 ++ February 3 ++	27	
3	M	25	1 day	i	LLL	17 090 P 83%	103	February 6	February 8	February 9 00 February 12 00 February 14 00	31	
4	F	42	1 day	iii	RML RLL	10 700 P 92%	104.4	February 10	February 13	February 20 ++	66.5	Developed a sterile pleurisy during resolution. Drug fever on twelfth day of treatment.
5	M	24	1 day	v	RUL	17 560 P 97%	103.8	February 11	February 19	February 13 00 February 16 00 February 19 00 February 21 00 February 23 00 February 26 00	74.5	Temperature normal on February 13 but rose the next day. Dosage increased to 12 grams a day with eventual good result.
6	F	58	3 days	vii	LLL	22 000 P 80%	103	March 4	March 6	March 6 00 March 8 00 March 11 00 March 13 ++ March 15 ++ March 18 ++ March 20 ++ March 25 00 March 26 00	34	Hypertensive cardiovascular disease. Auricular fibrillation. Some congestive failure. Drug fever on the tenth day of treatment.
7	F	68	1 day	xxiii	Central right mid lung	16,500 P 81%	104	March 6	March 7	March 13 00 March 15 ++ March 18 ++	30	
8	M	75	3 days	iii	RLL	16 000 P 90%	104	March 17	March 23	March 22 00 March 25 0+ March 29 ++	42	
9	F	57	1 day	iii	RUL RML	23 600 P 90%	104	March 17	March 24	March 25 ++	60	
10	F	51	3 days	vii	RUL	22 080 P 87%	105	March 19	March 21	March 19 00 March 23 00 March 26 00 March 29 00 April 1 00 April 3 00 April 5 00 April 8 00 April 11 ++	35	Apparently had drug fever from March 22 to March 26

TABLE 1—Continued

Case number	Sex	Age	Duration of disease before treatment	Type	Lobe	White blood cells	Temperature before treatment	Date treatment started	Date temperature normal	*Precipitin tests	Total dose sulfathiazole	Remarks
11	F	23	36 hours	I	LLL	20 720 P 81%	105	March 21	March 23	March 23 00 March 25 ++ March 27 ++ March 29 ++ April 3 ++ April 5 ++ April 8 ++	41	Severe diabetic. Type xx pneumonia in 1935. Type xiv pneumonia in February 1940.
12	M	17	12 hours	vii	LLL	18 000 P 92%	103.6	March 26	March 28	April 2 00 April 3 00 April 5 ++	23	
13	M	55	4 days	vii	LLL	21 320 P 88%	105	April 1	April 2	April 2 0+ April 5 ++ April 8 ++ April 10 00	43	
14	M	25	36 hours	v	RLL	27 600 P 94%	105	April 7	?	April 12 00 April 13 00 April 17 ++ April 20 ++ April 22 0+ April 24 00	74	It is uncertain when "essential recovery" took place here; fever remained between 101 and 102° until April 14 when it rose, reaching 104° on April 16. Drug was stopped, and temperature promptly fell. Patient also had sterile effusion.
15	F	53	2 days	xiv	LLL	21 280 P 86%	105	April 23	April 27	May 2 00 May 4 00 May 6 00 May 8 00	62	Rheumatic heart disease, auricular fibrillation, hypertension, diabetes mellitus. Had drug fever and rash on May 2.
16	M	31	3 days	I	LLL	23 680 P 96%	104	April 27	April 30	April 30 00 May 3 ++ May 6 ++	43	Had 6 grams of sulfapyridine before starting on sulfathiazole.
17	F	63	3 days	iv	RLL	27 840 P 85%	104	May 2	May 4	May 4 00 May 6 00 May 8 00 May 10 00		History of syphilis, partly positive serology, arteriosclerotic heart disease.
18	M	23	3 days	I	RLL	29 920 P 94%	105	May 3	May 4	May 6 00 May 8 00 May 10 00	27	
19	M	32	5 days	viii	RLL	32 680 P 90%	104.2	May 6	May 11	May 7 00 May 10 ++ May 13 ++	51	Had 7 grams of sulfapyridine before starting on sulfathiazole.
20	M	40	12 hours	I	RLL	22 250 P 88%	105	May 7	May 16	May 10 00 May 13 00 May 15 00 May 17 ++ May 20 ++ May 23 ++	82	
21	M	39	4 days	I	RML	21 280 P 88%	103.4	May 7	May 11	May 8 00 May 13 00 May 15 00 May 17 ++ May 23 ++	60	History of asthma.

* First column = two hour reading Second column = overnight reading

the time of appearance was accurately determined and its relationship to the beginning of normal temperature known, it may be said that in six the antibody appeared so close to the moment of 'essential recovery' as to indicate that it might be playing an important role in that process. In six other cases however, the antibody was not detected until about a week after the temperature had been normal, and in one case not until three weeks had elapsed.

DISCUSSION

It seems to us that the importance of these observations lies in the fact that a sharp difference is established between the response of patients treated with sulfathiazole and those treated with sulfapyridine when exactly the same technique of investigation is employed in both series. This difference may be expressed as follows: three-quarters of the cases of lobar treated with sulfapyridine recover " "

pearance of an excess of type-specific antibody in the blood serum as expressed by the precipitin reaction, whereas three quarters of those treated with sulfathiazole *do* show an excess of antibody. Moreover, when antibody does appear in the sulfapyridine-treated cases, it does so about a week after the temperature has become normal, whereas in about half of the sulfathiazole-treated cases it is first detected near the moment of "essential recovery."

In order to be certain that the facts are as stated, it is necessary to assure oneself that the two series of cases treated were similar. If, for example, one series had contained more very severe cases, or more cases in which treatment was begun late, or fewer examples of the higher types, such a series might be expected to show a higher percentage of antibody-formers. Such was not true of our sulfathiazole-treated group, there were no bacteremic cases, as opposed to two in the sulfapyridine group (both of which showed antibody, one being reported in the original paper). Treatment was actually begun, on the average, one day earlier in the sulfathiazole series than in the sulfapyridine. Lastly, the distribution of types was approximately the same. We therefore feel justified in concluding that a real difference exists between the responses of patients treated with the two drugs.

What is the most likely explanation of this phenomenon? It is theoretically possible that sulfapyridine *per se* might inhibit antibody-production to some degree. We have investigated this hypothesis fairly extensively during the past year by studying the antibody response in laboratory animals which were receiving chemotherapy. Heat-killed pneumococci and egg albumen have been used as antigens and, while the results are somewhat irregular, it is safe to say that neither drug can be shown to inhibit antibody-production. The other explanation, and to us the more probable, is that sulfapyridine is a somewhat more powerful antibacterial agent in human lobar pneumonia than is sulfathiazole, and that this difference expresses itself quantitatively in the pro-

portion of patients who show a greater activity of their immune mechanisms when treated with the newer drug. Additional support is lent to this hypothesis by the fact that the sulfapyridine-treated cases averaged 17 days of fever after treatment was begun, while those on sulfathiazole averaged 32 days.

SUMMARY AND CONCLUSIONS

1 Twenty-one cases of lobar pneumonia treated with sulfathiazole were studied for the appearance of an excess of type-specific antibody in the blood serum by means of the precipitin reaction with specific polysaccharide. Sixteen of these were observed to show an excess of antibody at the time the temperature became normal or thereafter.

2 As we had previously observed that about three-quarters of the patients treated with sulfapyridine did not show antibody, there appears to exist a greater stimulation of this immune mechanism in patients treated with sulfathiazole than in those treated with sulfapyridine.

3 This is interpreted as indicating that sulfapyridine is a somewhat more powerful antipneumococcal agent than sulfathiazole.

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RELATIONS OF EFFECTIVE RENAL BLOOD FLOW AND GLOMERULAR FILTRATION TO TUBULAR EXCRETORY MASS IN NORMAL MAN¹

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In 1938 Smith, Goldring and Chasis (12) described methods for measuring the "effective renal blood flow" (diodrast clearance) and the "tubular excretory mass" (diodrast T_m) by the excretion of diodrast at low and high plasma levels. The present report concerns the application of these methods to normal subjects under standard or quasi basal conditions, with special reference to the quantitative relations between the diodrast clearance, the inulin clearance and diodrast T_m in various subjects.

The subjects examined here were convalescent, volunteer patients of the Third (New York University) Medical Division of Bellevue Hospital, and were selected as carefully as possible to exclude renal disease. Clearance determinations were carried out in the morning without breakfast, although in a few instances a glass of orange juice was allowed. The subject was hydrated by the administration of one liter of water the night before, the water was measured out for him and he was allowed to take it *ad libitum* and without supervision. The next morning a second liter of water was taken between 7:00 and 8:30 a.m., and the subject was allowed out of bed momentarily. At about 9:00 a.m. an intravenous infusion containing the requisite concentrations of diodrast,

phenol red and inulin in 0.9 per cent saline was started, and urine collections were begun after a suitable washout period. Urine was collected by catheter the bladder being rinsed with saline at the termination of each urine collection period. Sterile technique was observed throughout.

DIODRAST CLEARANCE

It has been our custom after 3 or 4 periods during which the diodrast clearance has been measured at low plasma levels of diodrast (0.8 to 2.0 mgm. per cent of diodrast iodine), to proceed to some observation on the action of a physiologic or pharmacologic stimulus upon the renal circulation or alternatively, to elevate the plasma level of diodrast to 15 to 50 mgm. per cent of diodrast iodine, in order to saturate the tubules and measure the "tubular excretory mass" T_m , the maximal rate of tubular excretion (diodrast T_m). The initial clearance periods obtained on a particular day and uncomplicated by any factor other than those specified we have averaged into a single datum which we designate here as the diodrast clearance C_D . Thus the data on C_D recorded here (Tables I and II) for a particular date represent the average of 3 or more consecutive clearance periods. We feel that 3 such consecutive periods are the least upon which a trustworthy average figure can be based.²

DIODRAST T_m

For the measurement of diodrast T_m (Tables I, II and III), the plasma level of diodrast must

² Chesley and Chesley (2) have misinterpreted our preliminary reports, in that they have suggested that we have included in our standard observations periods when sodium sulphate was being used to maintain the urine flow in special but such data are not included here.

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The authors are indebted to the Winthrop Chemical Company for generously supplying some of the diodrast used in the later observations and to Messrs. Hynson, Westcott and Dunning for the preparation of phenol red in sterile 10 per cent solution. They also wish to express their appreciation of the continued interest of the Pfanzstiel Chemical Company in the preparation of pure inulin. Recently the U. S. Standard Products Company, Woodworth, Wisconsin, with the co-operation of Dr. Alf S. Alying have made physiologically tested inulin available in sterile ampules.

be raised to a point where the tubules are excreting this substance at a maximal rate (Under these conditions the diodrast clearance as ordinarily defined (UV/P) has, of course, no direct physiological significance) The rate of tubular excretion of diodrast, T_D , is calculated as the difference between the total quantity excreted per minute and the quantity excreted by filtration, $i.e.$,

$$(1) \quad T_D = U_D V - P_D C_{IN} F W$$

$$= \left(\frac{C_D}{C_{IN}} - F W \right) P_D C_{IN},$$

where U_D and P_D are the concentrations of diodrast iodine in mgm per cc of urine and plasma, respectively, V , the urine flow in cc. per minute, C_{IN} and C_D the inulin and diodrast clearances in

TABLE I
Diodrast clearance, etc., in normal men
(Columns 5, 6, 7, 8 and 11 corrected to 1.73 sq. m.)

Subject	Age	Surface area	Date	Plasma clearances			Effective blood flow	Filtration fraction	Rectal temperature	Diodrast T_m
				Inulin	Phenol red	Diodrast				
	years	sq. m.		cc per minute	cc per minute	cc per minute	cc per minute	per cent	° F	mgm iodine per minute
A M	31	1.69	April 9, 1937	122		664	1107	18.4		
J J		1.73	May 28, 1937	128	395	778	1325	16.5		
H C		1.70	May 28, 1937	122	375	718	1177	17.0		
F S	22	1.84	November 23, 1937	144	447	956	1738	15.1		
			November 29, 1937	144	429	876	1622	16.4		
T S	27	1.76	October 27, 1937	110	370	650	1205	16.9		
J W	50	1.69	November 17, 1937	111	294	575	967	19.0	98.5	39.0
V C	28	1.82	January 12, 1938	156	543	993	1623	15.7	100.5	51.2
			January 28, 1938	145	469	884	1535	16.4	98.5	55.0
			February 18, 1938	150	403	720	1334	20.8		
			February 23, 1938	132	418	842	1559	15.7		
			March 7, 1938	141	515	986	1780	14.3		
J Ho	50	1.78	February 4, 1938	112	293	536	891	20.9	98.5	45.1
J Cw	38	1.72	March 28, 1938	101	243	444	765	22.8		
			April 8, 1938	97	165	415	692	23.4		
			April 18, 1938	77	263	409	660	18.8		
			April 25, 1938	82	257	323	521	25.4		
			May 2, 1938	100	243	471	785	21.2		
P V	68	1.70	March 30, 1938	134	390	637	1143	21.0		
			May 11, 1938	111	346	680	1192	16.3		
P M	18	1.86	April 22, 1938	136	349	834	1604	16.3		
P F	32	1.62	April 29, 1938	136	524	955	1590	14.2		
			May 6, 1938	144	494	956	1542	15.1		
			May 19, 1938	139	470	852	1352	16.3	99.0	57.6
J Cs	36	1.61	April 6, 1938	135	320	555	1005	24.3		
			April 12, 1938	109	364	604	1079	18.0		
			April 15, 1938	113	395	625	1077	18.1	98.2	43.0
C J	24	1.70	April 13, 1938	178	471	818	1525	21.8	98.2	72.0
			April 20, 1938	179	526	826	1432	21.7		
D M	49	1.63	April 28, 1938	161	445	687	1185	23.4		
			May 4, 1938	136	412	693	1200	19.6		
B L	27	1.83	May 9, 1938	117	381	831	1510	14.1		
			May 13, 1938	118	369	705	1348	16.7		
			May 23, 1938	121	410	727	1320	16.6		
J Ca	43	1.38	May 18, 1938	146	532	795	1237	18.4		
			May 27, 1938	140	514	841	1342	16.6		
S M	35	1.67	May 16, 1938	116	363	716	1285	16.2		
J Ja	28	1.63	October 13, 1938	163	542	878	1394	18.6	98.6	58.3*
N B	49	1.78	November 8, 1938	126	397	555	925	22.7		
L D	39	1.78	December 1, 1938	145	525	885	1525	16.4		
G A.	47	1.65	December 2, 1938	140	436	624	1095	22.4		
B G	62	1.56	October 24, 1938	112	314	498	844	22.5		
			November 3, 1938	114	355	542	874	21.0		
			November 18, 1938	98	300	565	932	17.3		
D K.	48	2.08	November 4, 1938	89	351	509	955	17.5		
M S	26	1.62	October 25, 1938	90	278	529	860	18.2		

TABLE I—Continued

Subject	Age	Surface area	Date	Plasma clearances			Effective blood flow	Filtration fraction	Rectal temperature	Diodrast T_m
				Inulin	Phenol red	Diodrast				
	years	sq m		cc. per minute	cc. per minute	cc. per minute	cc. per minute	per cent	F	mg. iodine per minute
M. R.	54	1.73	November 16 1938	76	199	343	746	22.1		
W. G.	30	2.06	November 23 1938	131	401	780	1622	16.8		
R. D.	28	1.98	December 11 1937	131	452	713	1145	18.4		
			January 3 1938	125	519	719	1110	17.4		
			January 21 1938	134	550	758	1156	17.7		
			January 31 1938	139	498	768	1174	18.1		
			February 14 1938	131	472	718	1106	18.2		
			February 28 1938	131	448	684	1060	19.2		
			December 12 1938	151	542	742	1160	20.3		
			December 28 1938	155	592	843	1380	18.4		
			February 6 1939	150		906	1350	16.6		
			February 24 1939	199						
F. A.	45	1.85	December 9 1938	111	376	662	1050	16.8	99.2	56.8*
			December 29 1938	121		846	1340	14.3		
			January 30 1939	113		746	1268	15.2	98.6	53.7
			February 8 1939	109		743	1294	14.7		
E. Mc.	50	1.84	September 26 1938	125	390	624	1023	20.0		
			October 18 1938	132	359	600	953	22.0		
			October 28 1938	134	375	596	977	22.5		
			November 1 1938	135	395	663	1087	20.3		
			November 7 1938	119	375	541	846	22.0		
			November 14 1938	113	345	601	974	18.8		
			November 21 1938	117	373	612	1033	19.1		
			November 28 1938	128	341	579	958	22.1		
			December 14 1938	114	336	599	1010	19.0		
			January 4 1939	111		510	836	21.8		
			February 1 1939	119		496	800	24.0	100.0	45.1*
J. Co.	40	1.62	December 27 1938	140		764	1250	18.3	100.9	61.0
W. S.	48	1.52	February 3 1939	138		647	1085	21.3		
P. Mc.	54	1.86	November 17 1939			602	1027			
			November 21 1939	153		730	1200	21.0		
			November 29 1939	135		804	1312	16.8	98.7	54.4
E. R.	39	1.87	January 8 1940	116		582	965	19.9	98.8	47.2
J. F.	40	1.59	January 5 1940	108		527	868	20.5	99.3	34.6
T. W.	51	1.83	January 15 1940	166		716	1341	23.2	98.3	58.1
S. F.	31	1.91	January 19 1940	147		786	1381	18.7		
E. M.	24	1.76	January 22 1940	118		687	1338	17.2		
H. O.	17	1.73	January 24 1940	128		542	890	23.6		
			January 29 1940	149		680	1230	21.9	98.7	49.7
T. F.	50	1.90	January 12 1940	91		625	1042	14.6		
			January 26 1940	101		449	778	22.5	98.5	38.8
			February 2 1940	88		501	918	17.7		
H. S.	38	1.73	January 31 1940	168		762	1177	22.0	99.0	62.4
			February 5 1940	160		915	1418	17.4		
J. B.	28	1.94	February 7 1940	114		738	1222	15.5		
			February 21 1940	125		721	1174	17.3		
W. J.		1.81	February 14 1940	153		778	1278	19.7	98.6	56.9

Statistical summary entering each subject once only

Mean	130†	391	688	1189	18.9	53.3†
σ	21.7	85.5	135.3	242.4	2.4	9.1
σ_m	2.96	15.8	20.6	36.9	0.36	1.8
n	54	29	43	43	43	26

	Mean	σ	σ_m	n
Phenol red				
Inulin	3.1	0.34	0.063	29
Diodrast clearance				
Diodrast T_m	13.6	1.4	0.32	19
Inulin clearance				
Diodrast T_m	2.57†	0.28	0.055	26

* Diodrast T_m is average of observations on this day with other observations in Table II

† Includes subjects shown in Table III

 σ_m = Standard Error of Mean = σ/\sqrt{n} .

TABLE II

Diodrast clearance, etc., in normal women
(Columns 5, 6, 7 and 10 corrected to 1.73 sq m.)

Subject	Age	Surface area	Date	Plasma clearances		Effective blood flow	Filtration fraction	Rectal temperature	Diodrast T_m
				Inulin	Diodrast				
	years	sq m.		cc. per minute	cc. per minute	cc. per minute	per cent	° F	mgm. iodine per minute
A. G.	24	1.36	October 14, 1938	92	445	718	20.7	98.5	36.6
C. K.	30	1.67	November 8, 1939		441*	726*			
			November 20, 1939		595*	948*			
			November 28, 1939	140	654	1027	21.4	98.5	63.8
D. W.	22	1.72	December 1, 1939	145	722	1135	19.3	99.2	48.2
			December 8, 1939		782	1211			
M. Z.	26	1.84	December 11, 1939	125	628	970	20.0		
			December 18, 1939	113	584	863	19.4	99.4	46.5
E. R.	51	1.63	December 13, 1939	114	617	1094	18.5	98.8	42.7
A. P.	39	1.62	December 15, 1939	109	554	938	19.7	99.4	40.7
			December 22, 1939	102	538	871	18.0	99.3	39.3
C. B.	49	1.57	December 20, 1939	101	550	789	18.4	99.2	43.4
G. A.	30	1.40	December 26, 1939	147	708	1180	20.8		
C. S.	29	1.70	December 27, 1939	115	600	876	23.0	99.4	40.9
L. T.	29	1.69	January 8, 1940	122	606	1264	18.3	98.5	58.3
L. G.	26	1.57	January 17, 1940	105	559	954	18.8	98.8	

Statistical summary entering each subject once only

Mean	118.7	600.4	998	19.8	46.7
σ	17.5	87.0	162.7	1.4	8.5
n	11	11	11	11	9

	Mean	σ	n
$\frac{\text{Diodrast clearance}}{\text{Diodrast } T_m}$	12.8	1.62	9
$\frac{\text{Inulin clearance}}{\text{Diodrast } T_m}$	2.54	0.28	9

* Omitted from average.

cc of plasma per minute, F , the fraction of diodrast which is free and therefore filtrable, as determined by the plasma concentration of diodrast iodine and the plasma albumin content (see nomogram of Smith and Smith (13)), and W is taken approximately as 100—per cent of plasma protein/100

Albumin and total protein determinations were made by us on 57 occasions on 31 subjects, and it was found that FW averaged 0.72 ± 0.025 . In a subject with diodrast T_m of 52, inulin clearance of 125 cc and a plasma level of diodrast iodine of 20 mgm per cent, a deviation in FW of ± 0.02 makes only ± 1.0 per cent error in diodrast T_m . Wherever possible, however, protein determination should be made for diodrast T_m measurement in renal disease.

In the determination of diodrast T_m , the total "load" of diodrast carried to the tubules must, of course, exceed the maximal rate of excretion

if the tubules are to be saturated. The diodrast load/diodrast T_m ratio in our observations ranges from 1.5 to 5.0. Any ratio above 1.5 appears to be satisfactory for this measurement.³ We routinely take the average of 5 periods of ten to fifteen minutes each for this datum.

It early became clear that diodrast T_m , involving as it does a "saturation" phenomenon, or maximal excretory activity on the part of the tubules, is very susceptible to changes in body temperature. Observations made upon the same subjects at different temperatures indicate a temperature coefficient of about 2.0 for the excretory process, i.e., a rise of 1.0° F in body temperature increases the T_m value by about 10 per cent. Consequently, in all measurements of diodrast

TABLE III

Data on glucose T_m , etc., in normal men
(Columns 6, 8 and 9 corrected to 1.73 sq m.)

Subject	Age	Surface area	Date	Inulin plasma clearance	Rectal temperature	Glucose T_m	Diodrast T_m
	years	sq m.		cc per minute	° F	mgm per minute	mgm per minute
J. J.	28	1.65	March 15, 1939	162	98.6	370	56.4
J. Hu.	39	1.85	Feb. 13, 1939	128	98.4	322	
			Feb. 20, 1939	116	98.8	313	40.3
W. S.	48	1.52	Feb. 10, 1939	148	99.0	316	
A. L.	42	1.88	Feb. 17, 1939	131	99.6	254	
R. W.	23	1.79	Feb. 23, 1939	149	98.7	323	
A. Lo	41	1.95	April 12, 1939	157	99.0	380	
C. C.	42	1.77	May 15, 1939	181	98.4	405	64.3
			June 29, 1939	134	98.5	349	63.7
R. D.	28	1.98	May 24, 1939	150	98.0	336	
			June 9, 1939	117	98.0	368	59.8
H. M.	34	1.81	June 15, 1939	117	97.8		51.8
			June 2, 1939	150	97.8	461	
J. B.	45	1.48	June 14, 1939	138	98.2	440	65.7
			June 21, 1939	106	97.6	301	48.3
P. M.	36	1.72	June 28, 1939	127	97.7	331	59.7
			Oct. 11, 1939	93	97.8	243	55.3
T. S.	37	1.70	Oct. 16, 1939	119	97.8	264	62.4
			Oct. 23, 1939	114	97.8	275	59.4
C. P.	34	1.67	Oct. 18, 1939	156	98.2	354	57.5
			Oct. 25, 1939	138	98.2	327	57.0
E. Mc.	50	1.84	Oct. 20, 1939	152	99.0	397	58.2
			Oct. 27, 1939	161	98.2	433	56.7
			March 1, 1939	129	99.0	340	45.1
			Average	136		344	56.7

³ The load of diodrast carried to the tubules is the product of the plasma concentration and the renal plasma flow. It is assumed that the renal plasma flow remains the same during diodrast T_m measurement as in the control observations, hence we calculate the load as $P_D(C_D - FWC_{IN})$, i.e., the actual plasma concentration of diodrast during T_m measurement times the average diodrast clearance (C_D) observed at low plasma diodrast levels, minus the quantity of diodrast filtered per minute. Filtered diodrast is deducted in this calculation inasmuch as after diodrast has been filtered through the glomeruli it is no longer available for either tubular excretion or for saturation of the tubules.

T_m (as indeed in all our observations) the rectal temperature is recorded every twenty to thirty minutes. The rectal temperatures observed by us range from 97.5 to 99.5° F and average 98.5° F, and we have corrected all figures for diodrast T_m reported here to 98.5° F by adding or subtracting 10 per cent to the observed value for each degree of body temperature above or below this value.

On a very few occasions we have observed transient urticaria during diodrast T_m measurement. No serious disturbances have been encountered, although we have on numerous occasions elevated the plasma level of diodrast to 50 mgm. per cent of iodine or higher for periods of one hour or more.

PHENOL RED CLEARANCE

The phenol red clearance was followed until December, 1938, at which time it was abandoned in favor of other observations. Phenol red T_m has been reported in only one subject (12) and is not recommended.⁴

GLUCOSE T_m

Shannon and Fisher (9) have shown that, when the plasma concentration of glucose is elevated to a sufficient level, the rate of reabsorption of glucose by the renal tubules in the dog becomes maximal and constant. We here briefly report that we have found the same thing to be true in normal man, and include measurements of the maximal rate of tubular reabsorption of glucose, or glucose T_m in 14 subjects (Table III).

The rate of tubular reabsorption of glucose (T_a) is calculated as the difference between the total quantity filtered per minute and the quantity excreted in the urine, i.e.

$$(2) \quad T_a = P_G C_{IN} - U_G V$$

where C_{IN} is the inulin clearance, P_G and U_G are the concentrations of glucose per cc. of plasma

and urine, respectively, and V is the urine flow in cc. per minute. In order to raise T_a to its maximal value (glucose T_m) (i.e., to saturate all tubules whose glomeruli are maximally or nearly maximally active), the load of glucose delivered to the tubules should exceed the maximal rate of reabsorption by at least 20 per cent, i.e., the glucose load/glucose T_m ratio should exceed 1.20. The load of glucose carried to the tubules is the product of the plasma concentration and the rate of glomerular filtration in cc. of plasma, i.e., $P_G C_{IN}$. At high plasma levels of glucose, slight errors in the determination of plasma glucose or the inulin clearance introduce disproportionately large errors in the calculation of glucose T_m and maximal accuracy is attained at a load/ T_m ratio of 1.4 to 1.6. Glucose T_m should be determined with a rising or constant plasma glucose concentration, since the reabsorptive process exhibits an unexplained hysteresis (Shannon and Troast, personal communication).

The measurement of glucose T_m is valuable for two purposes. First, it affords a measure of the quantity of intact tubular reabsorptive tissue, or the tubular reabsorptive mass of the kidney, in contrast to the "tubular excretory mass" as measured by diodrast T_m . Second, it affords a method of evaluating the number of "active" nephrons and hence open glomeruli, since at an appropriate plasma level of glucose a critical decrease in filtration rate in a significant number of glomeruli will cause the connected tubules to become unsaturated, and glucose T_m will decrease by a corresponding amount (7). The application of glucose T_m in appraising glomerular activity will be discussed elsewhere, here we wish to report only the observed values under standard conditions.

The elevation of plasma glucose to the high levels involved in this measurement (350 to 700 mgm. per cent) may be expected to expand the plasma volume, under which conditions the renal blood flow might be modified either reflexly or through autonomous local adjustments. Evidence on this point will be presented separately, we need only note here that we do not consider the data on the diodrast clearance when obtained simultaneously with the measurement of glucose T_m as physiologically comparable with the other

⁴ Phenol red T_m measurement requires plasma concentrations of 25 mgm. per cent, at which level the skin discoloration is disturbing to the patient moreover phenol red, when given intravenously in large doses in dogs frequently causes nausea and a marked fall in the filtration rate, and if used for this purpose in these or other animals should be given subcutaneously or intramuscularly.

data on this clearance presented here. Consequently, the diodrast clearance has been omitted from Table III. We have no reason, however, to believe that either the inulin clearance or the value of diodrast Tm is altered in normal subjects under these conditions, and we consider these data (Table III) as physiologically homogeneous with those presented in Tables I and II and have accordingly included them in the statistical summary.

THE QUESTION OF PHYSIOLOGICALLY ACCEPTABLE "STANDARD" VALUES OF THE DIO- DRAST CLEARANCE⁵

Inulin, phenol red and diodrast

The first point to be considered in this connection is the possibility of hyperemia induced by *pyrogenic inulin*. The presence of pyrogen in inulin and a method of removing the pyrogen were reported by Goldring and Smith in 1936 (5), and a more effective method of purification was reported by Smith, Chasis and Ranges in 1938 (11). In adequate doses this pyrogen can produce renal hyperemia (10). Ever since the hyperemic properties of this pyrogen were discovered, we have been aware of the danger of this action complicating our observations. That such has not been the case we are convinced for the following reasons. When pyrogenic inulin is given in even threshold doses, the first reaction is a rise in temperature two to four hours after administration, usually with complaints of chilliness and other subjective reactions. We have never succeeded in producing renal hyperemia by doses too small to produce the febrile reaction. When administered in larger doses, there has, in our experience, invariably been a latent period of at least ninety minutes before the development of renal hyperemia. Since 1936 rectal temperatures have been taken routinely on all subjects, both during observation and every hour thereafter until 4 00 p m, and in all the data on diodrast clearance recorded here, the observations have been concluded within approximately sixty minutes after the first inulin injection. In no instance has the afternoon

temperature exceeded 100° F, nor have there been subjective evidences of pyrogenic reaction. Consequently, we set aside as improbable the possibility that our mean value for the diodrast clearance has been elevated by pyrogenic hyperemia.

With regard to *diodrast*, it is known that the rapid intravenous administration of relatively large doses of this substance cause a transitory fall in blood pressure (3), but the doses involved exceed many times the quantities which we use in clearance determinations. These quantities are typically 350 mgm (1 cc) as a priming dose, followed by infusion at the rate of 16 mgm per minute. Smith, Goldring and Chasis (12) found that small doses of diodrast (0.35 to 1.5 grams) do not increase the phenol red clearance, and more recently we have made numerous observations in which the plasma concentration of diodrast has been slowly elevated from 1.0 to 10 mgm per cent, without any increase in diodrast clearance. On the basis of this evidence we exclude the supposition that diodrast has produced renal hyperemia.⁶

With regard to *phenol red*, Herrick, Mann and Sheehan (6) have reported that injections of this substance increase the renal blood flow (thermostromuhr method) in unanesthetized dogs. The average dose used by these investigators was 15 cc of a 6 per cent solution injected in one minute, or 900 mgm per minute. In one of the two experiments illustrated by them the disturbance in renal blood flow had passed off in ten minutes, and in the other there was practically no change. In both experiments the dog vomited immediately after the injection. The largest quantity of phenol red we have used has been 30 mgm per minute for ten minutes as a priming dose completed twenty minutes before the first clearance period, followed by infusion at 4 mgm per min-

⁵ The question of quantitative correspondence between the plasma diodrast clearance and the actual renal plasma flow (or between the whole blood diodrast clearance and renal blood flow) will not be discussed here since it will be treated in a forthcoming publication.

⁶ This applies only to the measurement of the diodrast clearance at low plasma levels of diodrast. Following the injection of 30 to 40 cc. of diodrast for the measurement of diodrast Tm , there appear to be occasional disturbances in the renal blood flow, as indicated by a slight to moderate fall in the inulin clearance. The circulatory effects of these large doses cannot be examined by the diodrast clearance method, since the tubules are then saturated and the extraction ratio depressed. But any circulatory effects of large doses given relatively quickly would not controvert the evidence that small doses have no such effect.

ute (12), assuming a 10 kgm dog and 60 kgm man, the dose given by Herrick, Mann and Sheehan is about 180 times our priming infusion, and 1350 times our maintenance infusion. In the absence of better evidence, we therefore discount the possibility of phenol red having induced renal hyperemia.

PSYCHOGENIC DISTURBANCE, SELECTION, AND HYDRATION

In brief, we believe that the chemical elements involved in clearance determinations have introduced no significant error in the data of Tables I and II. We are, however, aware of three factors which should be considered in presenting our data as "standard" normal values.

First, there is the factor of *psychogenic* disturbance. The mere procedures of catheterization, venipuncture, and the attendance of 3 or more persons are obvious sources of apprehension to some patients. The actuality of psychogenic vasoconstriction in the kidney has recently been demonstrated (10) and, though the question is not open to experimental investigation at the present time, we fully recognize the possibility that such apprehension as may unavoidably be associated with the procedure of clearance determination may produce renal vasomotor disturbance. Where our subjects have been examined on 2 or more occasions the diodrast clearance has been larger in the second than in the first examination less than 50 per cent of the time (Tables I and II). Nevertheless, nearly all our data involve repeated observations on "trained" subjects: *i.e.* subjects who are quite accustomed to clearance examination, and this fact may have led to a figure for the mean diodrast clearance somewhat above the figures which have been reported by others (2, 17).

Secondly, we recognize the possibility that *selection* may have influenced our data. Many of our subjects have been selected with a view to the examination of pharmacologic agents, etc., and it may be that we have selected a group of subjects which, in respect to renal function, is not fairly representative of the general population. We wish merely to call attention to this possibility for we strongly discount its actuality.

The third factor bearing on this point is the

degree of *hydration* of the subject. Our subjects take a liter of water the night before, and another liter on the morning of the examination (the last water at least ninety minutes before the first clearance period) and, although we have observed no difference in a few comparative observations on the same subjects, it may be that this thorough hydration results in an increased renal blood flow. But even if such is the case, we believe that a standard technique such as this is preferable to one which will permit variable dehydration to enter as a complication.^{*}

In view of the above considerations, we present our data as "standard" rather than "basal" observations, emphasizing that the conditions under which they have been made are both physiological and reproducible. The technique of making all such observations must be improved as knowledge of the renal circulation is increased.

DISCUSSION

The larger series of data presented in Table I requires no discussion other than to note that the average inulin and phenol red clearances compare favorably with the average values previously reported for 25 subjects (12). The present average diodrast clearance is substantially less than that previously reported, but the previous figure was based on only 6 subjects 2 of whom were counted twice.

The most significant aspect of the present data is the quantitative relationship which exists between the diodrast clearance and the inulin clearance, on the one hand, and diodrast T_{in} on the other. Examination of the data on those subjects in whom diodrast T_{in} has been measured shows that this term has a close positive correlation with both clearances, as shown in Figures 1 and 2.

^{*} We recommend thorough hydration in order to maintain the urine flow and to permit accurate clearance periods. Where both hydration and Na_2SO_4 are contra-indicated, 7 to 8 per cent mannitol or sorbitol (Abbott ampules) may be incorporated in the infusion as a diuretic. Dr. Catherine Welsh and her co-workers (personal communication) have used the hexitols for this purpose in clearance studies on normal pregnant women and women with pre-eclampsia.

^{*} The ambiguity of the term "basal" is illustrated by the recent studies of Scott, Bazett and MacKie (8) on the circulation under various climatic conditions.

TABLE IV
Statistical analysis of subjects with diodrast T_m
(Men and women are treated as one series)

	Number of subjects	Range	Mean	σ	r	$\frac{\sigma}{\text{mean}} \times 100$
Diodrast whole blood clearance, cc per 173 sq m per minute	28	718-1566	1115	219		19.6
Diodrast plasma clearance, cc per 173 sq m per minute	28	455-921	669	128		19.1
Inulin plasma clearance, cc per 173 sq m per minute	35	92-179	131	22.1		16.9
Diodrast T _m , mgm iodine per 173 sq m per minute	35	36.6-72.0	51.6	9.4		18.2
<u>Diodrast whole blood clearance</u> Diodrast T _m	28	16.0-29.5	22.3	2.70	0.77	12.1
<u>Diodrast plasma clearance</u> Diodrast T _m	28	10.2-16.7	13.4	1.4	0.74	10.5
<u>Inulin clearance</u> Diodrast T _m	35	2.09-3.12	2.56	0.28	0.77	9.0

$$\sigma = \sqrt{\frac{\sum(m - \bar{x})^2}{n}} \quad r = \frac{\frac{\sum xy}{n} - \bar{m}_x \bar{m}_y}{\sigma_x \sigma_y} \quad \sigma_m = \frac{\sigma}{\sqrt{n}}$$

The value of r in column 6 refers to the correlation between the numerator and denominator

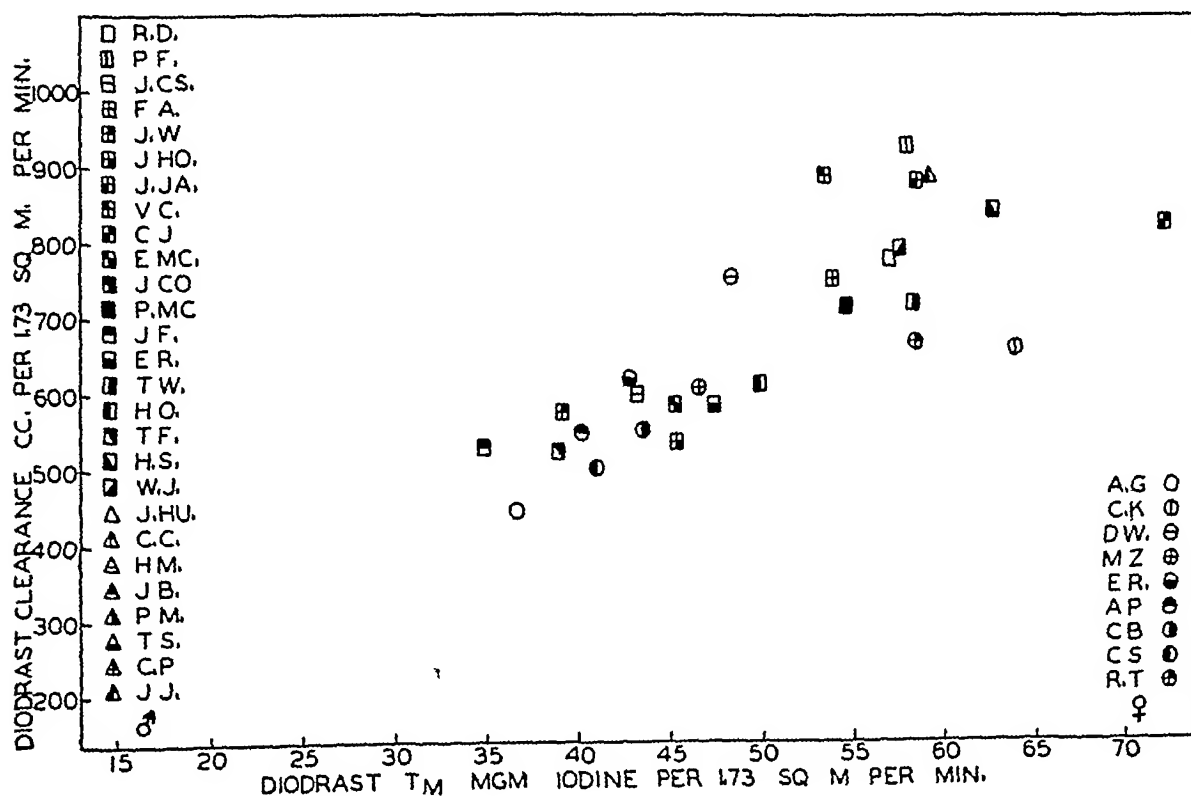


FIG 1 THE PLASMA DIODRAST CLEARANCE (EFFECTIVE RENAL PLASMA FLOW) IN 28 SUBJECTS (MEN AND WOMEN) IN RELATION TO DIODRAST T_m (TUBULAR EXCRETORY MASS), AS RECORDED IN TABLES I AND II

Each subject enters once only into the calculation of the correlation coefficient (0.77). Diodrast T_m may be taken as proportional to the total functional tubular tissue, and therefore closely proportional to the kidney weight.

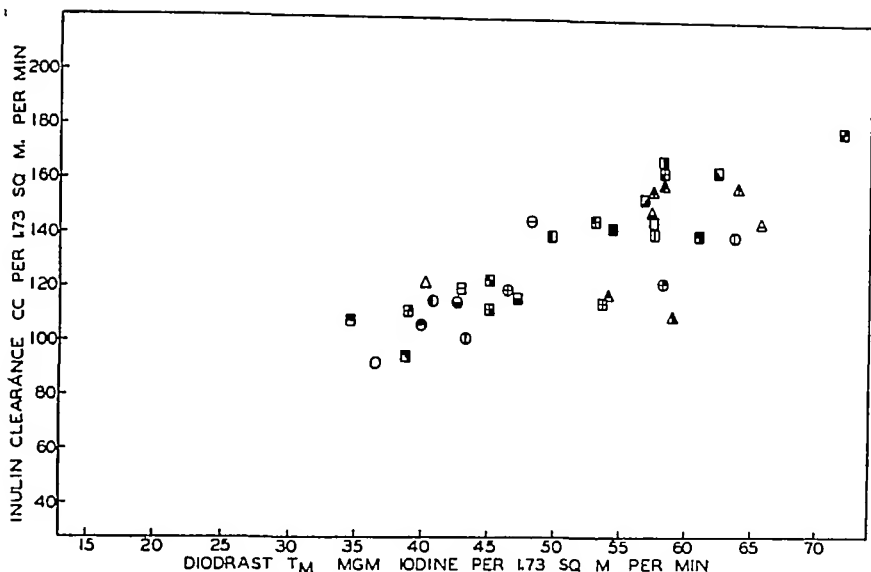


FIG. 2. THE PLASMA INULIN CLEARANCE (FILTRATION RATE) IN 35 SUBJECTS (MEN AND WOMEN) IN RELATION TO DIODRAST T_m (TUBULAR EXCRETORY MASS) AS RECORDED IN TABLES I II AND III

The correlation coefficient is 0.77

In Table IV we have calculated the coefficient of correlation between diodrast T_m and the whole blood diodrast clearance, the plasma diodrast clearance and the inulin clearance. There are also presented in this table the standard deviations for these clearances, calculated on the same subjects (Each subject is here considered once only). Data are available on 35 subjects for the inulin clearance, but on only 28 subjects for diodrast clearances since on 7 subjects diodrast T_m was determined simultaneously with glucose T_m , under which conditions, as we have pointed out above, the diodrast clearances are physiologically suspect.)

Reference to Table IV shows that the standard deviation expressed in per cent of the mean is substantially smaller when the clearances are related to diodrast T_m than when they are considered independently. We interpret this fact to indicate that differences in the clearances are in part attributable to such differences in the size of the kidneys as are not excluded by the usual correc-

tion for body surface area, these differences in kidney size being evident in data on normal kidney weights (16). To what extent diodrast T_m is strictly proportional to kidney weight is at present unknown, but since diodrast T_m is in theory a measure of the total quantity of tubular excretory tissue in the kidney, we would expect the correlation between these two terms to be of a significantly high order. In this view diodrast T_m may be taken as a measurement of kidney size, and from the close correlations shown in Table IV and Figures 1 and 2, we would be permitted to say that the whole blood or plasma diodrast clearance and the inulin clearance in various individuals are relatively more constant when compared per unit of kidney weight than when compared per sq m of body surface area.

The present series of data on glucose T_m is too small to afford an accurate statistical analysis. The average glucose T_m /diodrast T_m ratio in 10 subjects is 6.49.

Relative to the data in Table IV, it should be

noted that according to statistical theory it is impossible to define the absolute range of "normal" values. Statistical analysis at best affords a statement of the probabilities that a given observation belongs to the population which has been analyzed. If in this population there is a symmetrical distribution of the individuals about the mean, then 95 per cent of the individuals will fall within $m \pm 2\sigma$, and 99.5 per cent within 3σ . Or, conversely, there is one chance in 20 that an individual actually belonging to this population may fall outside the range of $\pm 2\sigma$, and one chance in 200 that it may fall outside the range of $\pm 3\sigma$. If, for example, a particular diodrast clearance falls just below 413 or just above 925 ($\pm 2\sigma$), there is still one chance in 200 that it is "normal" with respect to the data of Table IV. The absolute identification of this subject as having abnormal renal function is statistically impossible.

Thirty-five subjects is, moreover, so small a statistical series that it affords a fair chance of sampling error. We would, at the present time, place more emphasis upon the physiological sources of variation discussed above than upon the statistical significance of this limited series.

We can briefly record here that we have utilized the ballistocardiographic method of Starr, *et al* (15) for the measurement of cardiac output before and during the clearance determination in 6 subjects (12 observations). In no case have we found an increase in cardiac output, as referred to a control measurement on the morning of observation, to be associated with the clearance procedure (infusion, catheterization, etc.). This investigation is being extended by other methods of measuring cardiac output in order to confirm the accuracy of the ballistocardiographic method in our hands.

SUMMARY

Data on the diodrast and inulin clearances, and on the maximal rate of tubular excretion of diodrast (diodrast T_m) and the maximal rate of tubular reabsorption of glucose (glucose T_m) are presented for a series of normal men and women.

Diodrast T_m , a measure of the total quantity of tubular excretory tissue in the kidney, is conceived to be roughly proportional to the total quantity of renal parenchyma.

Statistical analysis of the data reveals that both the diodrast and inulin clearances increase in proportion to diodrast T_m , and that comparison of clearances in different individuals is more accurate when made on the basis of diodrast T_m than when the absolute values of the clearances are considered alone. This method of comparison is functionally equivalent to expressing the renal plasma or whole blood flow and the filtration rate on the basis of unit mass of renal parenchyma.

The significance of the mean values of the diodrast clearance, etc., is discussed in relation to the possible perturbation of renal blood flow by factors involved in the clearance procedure, such as inulin and diodrast, and by selection of subjects, hydration and psychic influences. It is believed that the data accurately reflect the renal blood flow under "standard" conditions which closely approach the ordinarily accepted concept of basal conditions in the systemic and renal circulation.

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METHODS

Only important changes from methods previously described (12) are detailed below.

1 Infusion fluid. This is prepared in 0.9 per cent saline (Sterisol saline, Schering and Glatz, Inc.) and administered from an open infusion flask without precautions for maintaining temperature, the rate being timed by means of a Murphy drip chamber and regulated by means of a 4-inch tunnel clamp. Typical protocols follow.

Diodrast clearance. To 800 cc. of saline are added 12 cc. of 35 per cent diodrast solution (Winthrop Chemical Company) and 25 cc. of 10 per cent inulin solution (U. S. Standard Products Company). Immediately after the infusion is started (zero minutes), 1.3 cc. of diodrast solution and 15 cc. of inulin solution are injected into the infusion tubing as a priming dose. (A control blood sample (B_0) is drawn when venipuncture is first made.) U_0 is collected at 20', B_1 at 22', U_1 at 32', U_2 at 44', U_3 at 56', and B_2 at 54' or 58'.

Diodrast T_m . The infusion tubing is disconnected from the needle, the infusion fluid drained to 400 cc., 11.5 cc. of diodrast are added to each 100 cc. of infusion

left in the flask and mixed, and 26 cc. of diodrast are injected into the infusion tubing as a priming dose. The infusion is accelerated for a short time to empty the dead space of dilute diodrast the rate then being restored to 4 cc. per minute. Twenty minutes later (ca 79') U_m (second discard) is collected, B_2 is drawn at 81' U_1 at 91', U_2 at 103', B_3 at 113', U_3 at 115', U_4 at 127', U_5 at 139' and B_4 at 137' or 139'.

In the average normal subject the above infusion will give 10 mgm. per cent of inulin in the plasma, 1.5 mgm. per cent of diodrast iodine between B_1 and B_2 , and 30 mgm. per cent between B_3 and B_4 . If the clearances are approximately known, the appropriate quantities of inulin and diodrast can be related to the desired plasma iodine concentration in mgm. per cent (P_D) and body weight (kgm.) on the basis that the priming dose is distributed in approximately 20 per cent of the body weight and that at a constant rate of infusion, this rate will equal UV. That is the priming injection in cc. should equal $\frac{P_D \text{ kgm}}{70}$ cc., and $\frac{P_D C_D}{700}$ cc. of 35 per cent diodrast solution should be added to each 100 cc. of infusion. For T_{mD} the priming injection is calculated in the same manner and the sustaining infusion should contain $\frac{T_{mD} + P_D 0.2 T_{mD}}{70}$ cc. of diodrast solution since $UV = T_{mD} + P_D FW 2.57 T_{mD}/100$. With 10 per cent inulin solution the priming dose should be $\frac{P_{IN} \text{ kgm}}{40}$ cc. and each 100 cc. of infusion should contain $\frac{P_{IN} C_{IN}}{400}$ cc.

Glucose Tm. Glucose (50 per cent solution) to the extent of 18 per cent is incorporated in the infusion fluid and 25 grams of glucose are injected into the infusion tubing as a priming dose, and 25 grams of glucose (pure) are given *per os* at zero minutes and again about midway in the observations. Depending on glucose Tm this yields 350 to 750 mgm. per cent glucose in arterial blood. If the hand is warmed in hot water the venous blood from the veins of the hand or wrist may be used instead of arterial blood, there being no A-V difference at this level of hyperglycemia and constant blood glucose level. The blood glucose level should be constant or rising for this measurement. It has been our experience that subjects showed marked differences in capacity to store and utilize glucose. Since glucose storage and utilization seem usually to be accelerated after the administration of a glucose infusion for an hour or so it is most difficult to maintain the requisite plasma level of glucose during the second hour.

2. Analytical methods We have recently adopted the *inulin* method of Alving, Rubin and Miller (1) with the modification recommended by them (personal communication). It has been our experience that pure diphenyl amine is necessary in this method. We have found the material supplied by Pfanstiel Chemical Company and the G. Frederick Smith Chemical Company to be satisfactory. In order to conserve blood we have changed the technique slightly from the original description.

Glucose is removed from blood and urine by treatment with a yeast suspension which has been well washed (6 to 8 times). This suspension will keep for a week or longer in the icebox, but must be centrifuged and freshly suspended on the day of use. Two cc. of plasma are mixed with 6 cc. of ca. 20 per cent yeast suspension (of known hematocrit) in 16 X 125 mm. pyrex tubes. The mixture is occasionally agitated, and centrifuged after fifteen minutes at room temperature. From 2 to 5 cc. (typically 2 cc.) of the supernatant fluid are removed to a 50-cc. Erlenmeyer flask containing 5 cc. of water and 6 cc. of $ZnSO_4 \cdot H_2SO_4$ mixture prepared as described by Somogyi (14). This mixture is well agitated and 2 cc. of 0.75 N NaOH are added. The flask is closed with the finger and well shaken. The mixture is centrifuged in 20 X 150 mm. heavy walled pyrex tubes and filtered through a pledget of washed cotton. The total dilution should yield about 0.50 mgm. per cent of inulin in the filtrate. Five cc. of the above filtrate are transferred in duplicate to the special tube recommended by Alving *et al* and the inulin determination carried out with the diphenylamine reagent according to their specifications. Color is read in an Evelyn colorimeter using a 635 filter. The urine samples after dilution to a U/P ratio of approximately 1.0 are yeasted, precipitated and analyzed in the same manner as the plasma.

For the determination of the yeast plus plasma inulin blank, 2 cc. of plasma from B_1 plus 2 cc. of 5 mgm. per cent inulin are treated with 6 cc. of yeast, and 3 cc. are precipitated as above. This blank varies from 0.1 to 0.7 and usually falls in the lower range of these figures. Duplicate inulin standards containing 0.2, 0.5 and 0.8 mgm. per cent, as well as triplicate reagent blanks are included. Apart from preparing washed yeast and washing apparatus, one analyst when using an 8-tube, I. E. C. centrifuge, can complete duplicate determinations on 5 plasma and 8 urine samples, with one B_1 sample, in eight hours.

Glucose in plasma and urine is analyzed by the Folin (4) method, using the technique of a single standard as described by Smith, *et al* (12).

Iodine is analyzed by the Kendall method as modified by Smith, *et al* (12) except that 3 cc. instead of 5 cc. of plasma are now used for low plasma iodine levels (1 to 2 mgm. per cent). Two cc. of plasma are used for high plasma iodine levels. An automatic filling 2-cc. burette, graduated in 0.01 cc. (E. Machlett and Sons New York) placed in a well lighted cabinet with white interior has been found extremely useful for titration. Six plasma and 12 urine samples can easily be analyzed in duplicate in eight hours by one analyst. This method has repeatedly been demonstrated to give 100 per cent recovery of added iodine in organic form and yields very satisfactory checks. The chief difficulty encountered in its use has been in the growth of mold on the inner walls of the 5 gallon bottles which we commonly use for distilled water. This mold becomes detached and catalyzes the oxidation of KI to I_2 . The difficulty has been cir-

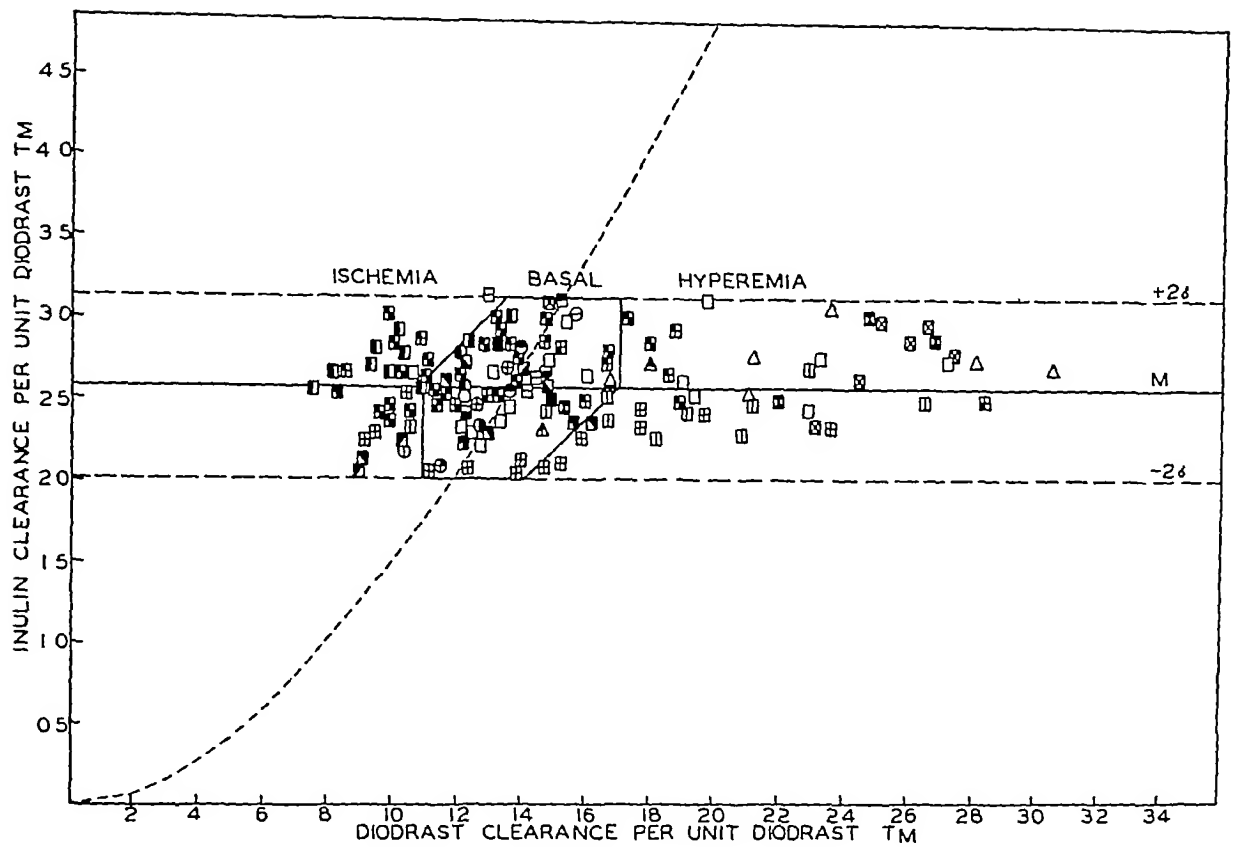


FIG 1 THE FILTRATION RATE (INULIN CLEARANCE) IN NORMAL SUBJECTS IN RELATION TO THE RENAL PLASMA FLOW (DIODRAST CLEARANCE) DURING BASAL CONDITIONS, ISCHEMIA INDUCED BY ADRENALIN, ETC , AND HYPEREMIA INDUCED BY TYPHOID VACCINE OR PYROGENIC INULIN

On the assumption that diodrast T_m , which is a measure of the total tubular excretory tissue in the kidney, is closely proportional to the total renal weight, a comparison of the inulin and diodrast clearances in terms of the respective value of diodrast T_m in various subjects is equivalent to comparing the filtration rate and renal blood flow per unit weight of kidney

Each datum represents the average of two or more clearance periods, each subject furnishing several datum on each examination under ischemia or hyperemia

The solid horizontal line shows the predicted relation if the changes in renal blood flow are attributable solely to changes in efferent resistance, the rising dotted line shows the predicted relation if the changes in blood flow are attributable solely to changes in afferent resistance, the alternate resistance in each case remaining constant at the status of the mean of the basal observations, 95 per cent of which are included in the hexagon

recorded in the above papers and similar observations, made by the same methods, which need not be presented in detail

The above data are presented graphically in Figures 1 and 2 Each datum referred to the "basal" group is the average of three or more consecutive clearance periods taken on any one day, each subject being recorded as often as he or she was examined Each datum referable to "ischemia" or "hyperemia" is the average of at least two successive clearance periods obtained during reduced or increased renal blood flow, each subject thus contributing several data from

each occasion on which ischemia or hyperemia was induced

Figure 1 presents the ratio C_{IN}/T_{mD} (the filtration rate per unit diodrast T_m) in relation to C_D/T_{mD} (the effective plasma flow per unit diodrast T_m) Instead of analyzing this miscellany of data on their intrinsic relations, we have arbitrarily chosen to appraise them in terms of our previously published standard figures Goldring, *et al* (8) have reported that in 35 men and women the average value of $C_{IN}/T_{mD} = 2.56 \pm 0.28$ cc per minute, for purposes of comparison we have marked this mean (solid line)

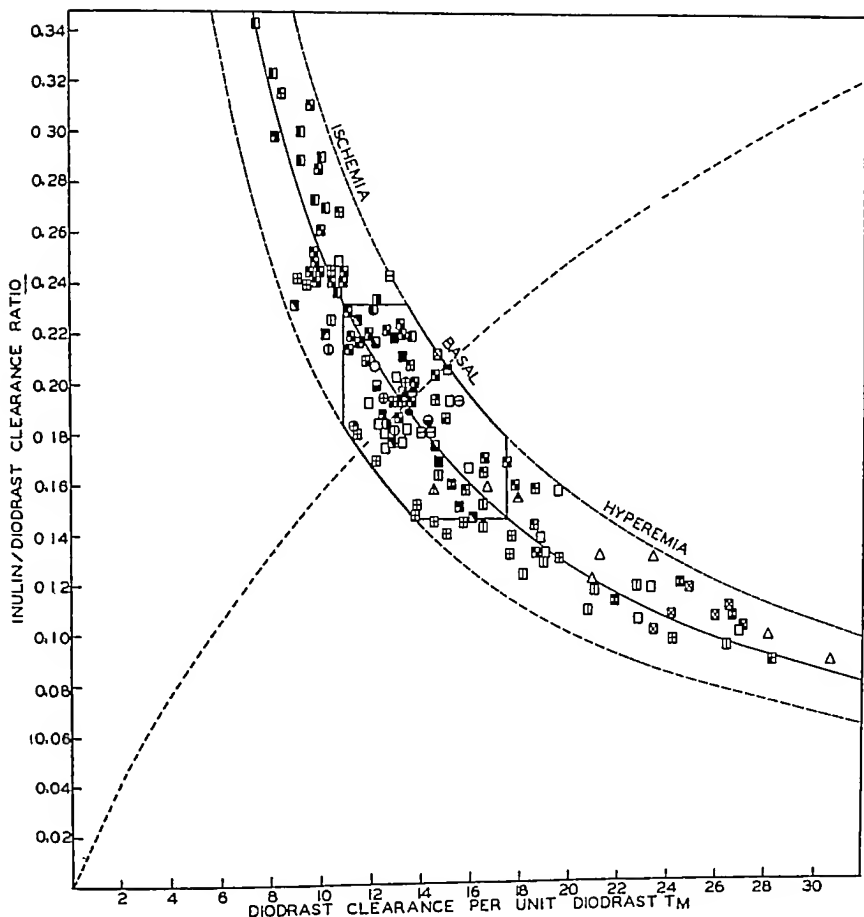


FIG. 2. THE DATA OF FIGURE 1 ARRANGED TO SHOW THE PER CENT OF WATER FILTERED OUT OF THE PLASMA (FILTRATION FRACTION) AS GIVEN BY THE INULIN/DIODRAST CLEARANCE RATIO AT VARIOUS RATES OF BLOOD FLOW

As in Figure 1 the solid line refers to changes in efferent resistance the dotted line to the changes in afferent resistance.

with twice its standard deviation (dashed lines) on the ordinates of Figure 1. It is to be noted especially that the mean value quoted from Goldring, *et al.*, includes only observations made under what we have here called "basal" conditions, and that in its calculation each subject

enters only once, thus contributing to the mean only his or her average behavior, while the data of Figure 1 comprise the unaveraged and hence more widely distributed behavior of all individuals, not only under basal conditions, but also during ischemia and hy

It is evident from this comparison that the miscellany of unaveraged values of C_{IN}/Tm_D obtained at all renal blood flows is still adequately contained within the statistical parameters of the more restricted "basal" data. It may be said, then, that the filtration rate per unit diodrast Tm in various normal subjects retains the same mean value during ischemia and hyperemia induced by the measures specifically stated above as it has in the basal condition. (It is not implied that this constancy will obtain if ischemia or hyperemia are induced by measures other than those stated.)

The view that this constancy of the filtration rate is to be attributed to the physiological circumstance that changes in renal blood flow are mediated through changes in the tonus of the efferent, rather than the afferent, glomerular arterioles requires that the effective glomerular filtration pressure must vary inversely as the renal blood flow. In the following discussion we shall inquire both whether the observed facts are open to any other interpretation, and if not, whether they are consonant with the application of simple hemodynamic principles to the glomerular circulation.

THE NOTION OF CONDITIONED FILTRATION

Although contrary to the accepted principles applicable to glomerular filtration in particular, and capillary permeability in general, it might be argued that the constancy of the rate of glomerular filtration at varying rates of renal blood flow is attributable to special properties of the filtering bed whereby the separation of capsular urine from plasma is independent of glomerular pressure. In considering such an hypothesis, it must be noted that in mammals we have no specific knowledge of the rate of passage of water *per se* across the glomerular membranes, our information being restricted to inulin and other solutes. It might be supposed, then, that the glomerular membranes so condition the passage of inulin that the quantity of this substance which enters Bowman's capsule per unit time is both constant and independent of glomerular pressure. That such conditioning does not apply to inulin molecules *per se* is controverted by the facts, first, that inulin is present in the

capsular fluid of frogs and necturi in the same concentration as it is present in plasma water (10), and second, that under controlled conditions in individual animals, inulin is excreted in widely varying total quantities, but always in proportion to its plasma concentration (7, 11, 14, 16, 19, 20, 23, 25), such wide latitude in absolute rate of excretion, combined with close proportionality to plasma concentration, is evidence against the conditioned passage of this molecule by the glomerular membranes.

It could still be argued, however, that the conditioning factors in the glomerular membranes are such as to permit the passage into Bowman's capsule per unit time of a constant *volume* of the solvent, water, with its inulin and all other contained solutes, the volume so passed being relatively independent of glomerular pressure. (Such a system is roughly imitated in a "slow" filter, *ie*, one in which a high resistance so restrains the movement of fluid that hydrostatic pressure is subordinate to other forces in determining the rate of passage.)

Against this interpretation the following evidence can be adduced. Any membrane conditioning the passage of water independently of pressure can do so only under circumstances where the gross movement of water is highly restricted, and where specific intermolecular forces (surface tension, adsorption, diffusion, solubility, etc.) are the determinants of translocation. Under these circumstances the passage of various molecular species are, with no known exception, differentially conditioned, at least to the extent that the more diffusible species move more rapidly than the less diffusible ones. The now abundant evidence on the passage of solutes through the glomeruli reveals no such differential permeability. The work of Richards and his collaborators (17, 18) has shown that all analyzable, diffusible solutes in the plasma are passed in unchanged proportions into the capsular space of the Amphibia, the most noteworthy instance being the simultaneous passage of water and inulin molecules, as referred to above. An equally strong argument can be based upon the identity of the inulin and creatinine clearances in the dog, frog and rabbit (7, 11, 19, 20, 22, 39), which identity is preserved under all conditions.

of filtration so far examined in normal animals. So far as man is concerned, more immediate evidence is available in the identity of the creatinine and inulin clearances in phlorizinized subjects (21), and the identity of the xylose and inulin clearances during hyperglycemia in dog and man (Shannon and Fisher, 24, and unpublished observations by these authors). Lastly, there are now available observations that show that the clearances of mannitol, sorbitol, and sorbitan in normal men and women, and in women with pre-eclampsia are identical with the simultaneous inulin clearance (28). Since the molecular species enumerated above differ markedly in diffusion velocities and in physical properties (2, 31), this evidence argues against the belief that the constancy of the filtration rate is due to the circumstance that the movement of water itself is restrained to a constant volume per unit time by the high resistance of the filter.

If the above evidence is adequate to exclude the conditioned passage of inulin specifically, and the conditioned passage of water and solutes generally, then the only alternative is to recognize that water with its contained solutes moves through the glomerular membranes in consequence of differences in hydrostatic pressure, not restrained to a significant degree by viscous or frictional forces. This view is, of course, the classical concept of glomerular filtration.

FILTRATION PRESSURE EQUILIBRIUM

We pass, then, to the consideration of the pressure relations within the glomeruli. It is recognized, first, that the passage of plasma water and diffusible solutes into Bowman's capsule occurs only in consequence of the fact that the hydrostatic pressure within the glomerular capillaries (P_G) exceeds the sum of the oncotic pressure (P_O) plus the capsular pressure (P_C). (By capsular pressure we designate the pressure existing in Bowman's capsule; this pressure will be approximately equal to the intrarenal or interstitial pressure and must, so long as urine is flowing, be sufficient to overcome the resistance offered by the tubules and collecting ducts to the passage of urine into the renal pelvis where, for purposes of discussion, the pressure may be taken to be zero.)

Secondly, in conformity with generally accepted relations in the mesenteric and systemic capillaries (12), we conceive that the filtration process is a reversible one and that if P_G falls below $P_C + P_O$, fluid will be reabsorbed from Bowman's capsule into the glomerular capillaries. Hence we write

$$(1) \quad P_G \xrightleftharpoons[\text{reabsorption}]{\text{filtration}} P_C + P_O$$

After a certain interval of exposure, an interval which will be determined by the permeability of the glomerular membranes, the opposing pressures tending to effect filtration and reabsorption, respectively, will become equal to each other and the process of filtration will stop. This state we designate as filtration pressure equilibrium, or more briefly filtration equilibrium. Under these conditions, the hydrostatic pressure in the glomerular capillaries (P_G) is equal to the sum of the equilibrium oncotic pressure (P_O) plus the capsular pressure (P_C).

$$(2) \quad P_G = P_C + P_O$$

This concept of filtration equilibrium will be made clearer by reference to Figure 3.

We will return later to the question of whether or not the duration of exposure of the plasma in the glomerular capillaries is sufficient to permit filtration equilibrium to be reached. But to facilitate discussion we will assume in the following that such is the actual case.

FILTRATION PRESSURE

On the question of what pressure is available in the glomerulus to effect filtration, Winton's (37, 38) studies on the isolated dog kidney indicate that the mean glomerular pressure in this preparation can vary from 30 to 90 per cent, and averages about 60 per cent, of the systemic pressure. If we take the more conservative figure of 50 per cent for the mean glomerular pressure, and if we take the mean systemic pressure as 90 mm of Hg, the mean glomerular pressure (P_G) will average 45 mm. As stated in equation (2), at equilibrium this value must be equal to the oncotic pressure plus the capsular pressure. Accepting the inulin/diiodrast clearance ratio as identical with the per cent of plasma

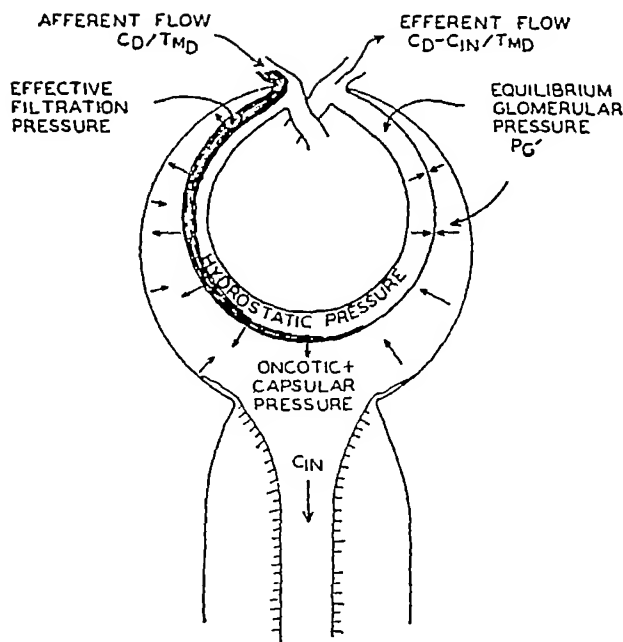


FIG 3 FILTRATION EQUILIBRIUM IN GLOMERULUS

It is supposed that in the arterial end of the glomerular capillary water and solutes move from capillary into capsule because the hydrostatic pressure (P_a) exceeds the sum of the oncotic pressure (P_o) and the capsular pressure (P_c). The pressure gradient between capillary and capsule, indicated by the shaded area, is dissipated in effecting the process of filtration and moving the blood forward in the capillary, so that by the time the blood reaches the efferent arteriole $P_a = P_c + P_o$, and the process of filtration stops. This condition is designated as filtration equilibrium. Insofar as the hydrostatic pressure suffers a further decrement in the distal portion of the capillary after filtration equilibrium has once been reached, capsular fluid will be reabsorbed, as in the systemic capillaries, leaving $P_a = P_c + P_o$.

Since P_o may be calculated from the initial oncotic pressure of the plasma (P_o) and the filtration fraction, we may, by taking P_c at an arbitrary and constant value, calculate P_a for various values of the renal plasma flow (diastolic clearance) and filtration rate (inulin clearance)

water filtered through the glomeruli, or what we have called the filtration fraction (FF), we may calculate the equilibrium oncotic pressure (P_o') as

$$(3) \quad P_o' = P_o \left(1 + \frac{FF}{10 - FF} \right),$$

where P_o is the initial oncotic pressure

FF averages 18.9 per cent in men and 19.8 in women, taking the round figure of 20 per cent, and taking P_o as 24 mm (36), at filtration equilibrium the plasma proteins will have been concentrated 1.25 times, and P_o raised from 24 to 30 mm Hg. Under these conditions the

capsular pressure ($P_c = P_a' - P_o'$) will be equal to 45 - 30 or 15 mm Hg. There is, at present, no way of determining the capsular pressure in man, but Winton (38) reports the interstitial pressure of the excised dog kidney to be typically below 15 mm Hg, and it may be inferred that the normal value in man, if not less than this, is probably not more than 25 mm.

Accepting tentatively the figure of 15 mm for the capsular pressure, and assuming that during constriction of the efferent arterioles P_a' rises to 70 per cent of the mean systemic pressure, or to 63 mm, then filtration will continue until P_o rises from 24 to 48 mm (63 - 15 mm), *i.e.*, until the plasma proteins have been doubled in concentration by the filtration of 50 per cent of the plasma water. A filtration fraction of 50 per cent lies well above the highest normal filtration fraction on record (33 per cent), so that it appears that ample pressure can be made available by efferent constriction to account for both the mean and the maximal filtration fraction observed.

Since at equilibrium, $P_a' = P_o' + P_c$, the last two terms set the maximal hydrostatic pressure available to propel blood through the efferent arteriole and the postglomerular circulation. Continuing with the above example, if 50 per cent of the plasma water is filtered, the hydrostatic pressure will be 48 + 15 = 63 mm, while if only 10 per cent of the plasma water is filtered, the hydrostatic pressure will be 26.6 + 15 = 41.6 mm. Both during ischemia and hyperemia, therefore, there will be sufficient hydrostatic pressure at filtration equilibrium to maintain the postglomerular circulation. If filtration equilibrium is not reached, P_a' will be greater than $P_o' + P_c$ and a still more effective pressure head will be available for the postglomerular circulation.

VELOCITY OF FILTRATION

Whether the permeability of the glomerular membranes is such as to permit filtration equilibrium to be reached before the blood leaves the glomeruli is not established. Richards and Walker (18) report that the glomerulus of the frog may filter water at the rate of 4 cubic mm per hour. The effective filtration pressure in the frog may be taken as 2.5 to 5 mm Hg (6, 9, 31).

With 2,465,600 glomeruli in two human kidneys (14), a comparable rate of filtration would yield 32 to 64 cc. per mm Hg effective filtration pressure and, since the initial glomerular pressure probably exceeds $P_c + P_o$ by several millimeters, the above figure exceeds the requirements of the largest observed filtration rate.

The total capillary surface area of a typical human glomerulus, according to Book (1), is 0.3813 sq mm, in the two kidneys the total filtering surface would then be slightly under 1.0 sq m. Taking the average renal plasma flow as 688 cc. per minute and the average filtration rate as 130 cc. per minute, it follows that some 12 cc. of plasma are passed per second over a filter which has a total area of about 1.0 square meter, giving a sheet of plasma virtually 0.012 mm deep (The filter itself is scarcely over 0.001 mm in thickness). The premise of filtration equilibrium requires that within one second's time and by the movement of 20 per cent of the water through the filter the pressures on both sides are equalized.

Landis (12) has calculated that an increment of 50 mm H_2O increases the rate of filtration through the mesenteric capillaries of the frog by 0.03 cubic micra per square micron per second. With a total capillary area of 1.0 meter, 50 mm of H_2O , or 3.68 mm Hg, would filter 4.74 cc. of water per minute. This figure is considerably below both the filtration rate recorded by Richards and Walker and the requirements of the human glomerulus, but it is not of an order of magnitude to cast serious doubt upon the possible velocity of filtration in the latter.

If filtration equilibrium is not reached in the distal portion of the capillary, the glomerular membranes fall in the category of a "slow" filter as described above, and it would be expected that there would be a differential passage of those solutes which have a much higher diffusion coefficient than does inulin (2). The evidence against such differential passage, as reviewed above, argues generally in favor of equilibrium. Although the thesis is not at present amenable to experimental proof in the human kidney, we are inclined, in the light of the available evidence, to accept it as a premise in the analysis of glomerular dynamics.

If filtration equilibrium is reached at normal rates of blood flow, it will also be reached during

ischemia, since greater time will be available for equilibrium to be attained. And since the maximal rate of blood flow in any one individual is but little over twice the basal, it may be assumed that filtration equilibrium will also be reached during renal hyperemia.

FILTRATION FRACTION IN RELATION TO RENAL BLOOD FLOW

In Figure 2 we have plotted the filtration fraction (calculated as the inulin/diodrast clearance ratio) against the diodrast clearance per unit diodrast T_m , or C_D/T_{mD} ³ and it will be seen that the filtration fraction is inversely related to the renal plasma flow—a statement which is merely a corollary of the fact (Figure 1) that the filtration rate is constant at all rates of renal plasma flow.

The inverse relationship of Figure 2 is described by the formula for a rectangular hyperbola wherein the product of the two terms, FF and C_D/T_{mD} , is a constant:

$$(4) \quad FF \frac{C_D}{T_{mD}} = k$$

Since

$$(5) \quad FF \frac{C_D}{T_{mD}} = C_{IN}/T_{mD},$$

it follows from (4) that

$$(6) \quad C_{IN}/T_{mD} = k$$

The value of k as given by the ratio C_{IN}/T_{mD} in the observed data is 2.56 ± 0.28 . Inserting this value in equation (4) yields the solid curve in Figure 2, while $\pm 2\sigma$ yields the dashed lines.

CHANGES IN GLOMERULAR PRESSURE AND BLOOD FLOW ATTRIBUTABLE TO CHANGES IN EFFERENT ARTERIOLAR TONE

In order to interpret the data of Figures 1 and 2, we pass to the consideration of what relation

³ It does not materially alter this analysis if the diodrast extraction ratio in the blood going to active nephrons is less than 1.0 by some constant amount or if a small fraction of the urinary diodrast is derived from the red blood cells (5, 33, 34). Appropriate correction for this circumstance can be made by taking the true plasma flow as C_D/E_D , where E_D is the arteriovenous diodrast extraction ratio in this blood. Substitution of this term in equation (4) will lead to proportional changes in all subsequent calculations but will not change the general dynamic relations. The maximal correction as indicated by the available data on E_D in the dog is about 10 per cent.

ships may be expected to obtain between C_{IN}/Tm_D , C_D/Tm_D and FF if changes in renal blood flow are the result solely of changes in resistance offered by the efferent arterioles, the resistance offered by the afferent arterioles and the postglomerular circulation and the mean systemic pressure remaining constant

In any simplified quantitative treatment, it is unnecessary to consider the whole blood diodrast clearance, since the plasma diodrast clearance serves equally well to describe volume flow changes so long as the relative volume of cells in the blood remains constant. We may, in general, speak of changes in blood flow, although actually all data and equations given here refer to plasma flow. We may also neglect changes in viscosity associated with the separation of glomerular filtrate, that such changes do occur is clear, but it is doubtful if they are normally of such a magnitude as to be important.

The following propositions are germane to the quantitative description of glomerular pressure

(a) The glomerular blood flow is represented, not by the afferent (or total) flow entering the glomerulus, but by the efferent flow leaving the glomerulus, assuming that there is no significant aglomerular blood supply to the tubules, the efferent flow is given by the difference between the total flow, C_D/Tm_D , and the volume of filtrate, C_{IN}/Tm_D , or $C_D/Tm_D - C_{IN}/Tm_D$

(b) The effective glomerular pressure (π_e , the pressure available to move blood between the glomeruli and the renal vein) is the difference between the hydrostatic pressure in the efferent end of the glomerular capillaries and such pressure distally as may oppose this hydrostatic pressure. Contributing to this opposing pressure is the renal venous pressure, a force so small (1 to 3 mm Hg) that for general purposes it may be neglected. But in addition to venous pressure, a force equal to the sum of the renal interstitial pressure (P_o) plus the oncotic pressure of the blood (P_o) opposes the perfusion of the kidney. The way in which the latter operates will be clear if it is noted that within any semipermeable compartment immersed in water at atmospheric pressure there exists a hydrostatic pressure relative to the atmosphere equal to the osmotic (or oncotic) pressure of the non-diffusible constituents, this hydrostatic pressure must be

maintained so long as water continues to exist outside the compartment, otherwise water will enter until equality between osmotic force and hydrostatic pressure is re-established. Conversely, in order to maintain water outside the compartment, the internal hydrostatic pressure must equal the oncotic pressure.

Applying the above principle to the kidney, if at any point in the capillary bed between the glomeruli and the renal vein an opportunity exists for osmotic equilibrium to be attained between the blood and the interstitial fluid, then the hydrostatic pressure of the blood in the peritubular capillaries must be at least as large as the oncotic pressure, P_o . Should the pressure be lowered at either end of the capillary, the internal hydrostatic pressure will move blood along the pressure gradient and simultaneously interstitial fluid will move into the capillary, and this process will continue until the ingress of interstitial fluid either restores the hydrostatic pressure to P_o , or until the available interstitial fluid is exhausted. Thus, so long as free interstitial fluid exists and so long as opportunity is afforded for the attainment of osmotic equilibrium between this fluid and the plasma, the hydrostatic capillary pressure cannot be less than P_o . Now in point of fact interstitial fluid is present around the peritubular capillaries throughout their length, and in view of the large expanse of these capillaries it must be assumed that osmotic equilibrium does exist along at least some portion of this capillary bed, consequently, the capillary pressure in this bed and at all points proximal to it cannot fall below P_o . (This is equivalent to saying that in consequence of the fact that water is free to move through the capillary wall, the capillary is compressed by an external force equal to the difference in vapor pressure of water on the two sides, π_e , the oncotic pressure. Once the blood moves into an impermeable vessel (vein) the hydrostatic pressure will fall below the oncotic pressure, since the ingress of water to maintain this pressure is no longer possible. The above argument will perhaps be clearer if, for such expressions as "osmotic equilibrium" or "the opportunity to attain osmotic equilibrium" there is substituted the idea of an instantaneous transfer of water to the

end that osmotic equilibrium is invariably maintained)

Since there also exists in the kidney an interstitial pressure (P_o) which compresses the capillary bed externally, this pressure must be added to P_o , so that the minimal capillary pressure must be written as equal to $P_c + P_o$. Only when the perfusion pressure exceeds $P_o + P_o$ will fluid move through the peritubular capillaries into the renal vein, short of this time any increment in perfusion pressure will serve only to move fluid out of the capillary into the interstitial space until osmotic equilibrium is attained.

The effective perfusion pressure, P_E is then the glomerular pressure, P_G , minus the opposed pressures, $P_c + P_o$, or

$$(7) \quad P_E = P_G - (P_c + P_o)$$

(c) Where a change in blood flow results from a change in the resistance offered by the efferent arterioles (both afferent arteriolar resistance and postglomerular resistance being held constant), the effective perfusion pressure, P_E , will according to hemodynamic principles vary inversely as the efferent blood flow or

$$(8) \quad P_E = k_1 \frac{1}{C_D/Tm_D - C_{IN}/Tm_D}$$

Hence, from (7) and (8),

$$(9) \quad P_G = P_c + P_o + \frac{k_1}{C_D/Tm_D - C_{IN}/Tm_D}$$

The above equation affords a hemodynamic description of how the efferent blood flow will vary with changes in glomerular pressure when the variable resistance is on the efferent side of the glomeruli

(d) But under the premise of filtration equilibrium, the glomerular pressure, P_G , as it appears in equation (9), is identical with P_o in equation (2) by substituting for P_o in this equation the right hand term of equation (3), and replacing FF by $\frac{C_{IN}/Tm_D}{C_D/Tm_D}$ of equation (6), it follows that

$$(10) \quad P_G = P_c + P_o + \frac{P_o C_{IN}/Tm_D}{C_D/Tm_D - C_{IN}/Tm_D}$$

Equating (9) and (10),

$$(11) \quad P_o C_{IN}/Tm_D = k_1$$

Since P_o in any one subject is in principle a fixed value,

$$(12) \quad C_{IN}/Tm_D = \text{a constant value.}$$

Thus it is deducible in theory that, when changes in renal blood are a result solely of changes in the resistance offered by the efferent arterioles, the rate of glomerular filtration will remain constant. This is the relationship which is demonstrated to exist in fact in the human kidney as shown in Figure 1.

Relative to equation (11), C_{IN}/Tm_D has the experimentally determined value of 2.56 if P_o is taken as 24 mm Hg (36), $k_1 = 61.4$. The inverse relations that exist between C_D and FF (Figure 2) issue, of course, directly from the constancy of the inulin clearance.

By arbitrarily taking $P_o = 15$ mm Hg (*vide supra*), and assuming that this term remains constant at varying rates of blood flow, and by taking $P_o = 24$ and C_{IN}/Tm_D as equal to 2.56 (as experimentally observed) P_G can be calculated from equation (10) for various values of C_D . Such calculated values are shown graphically in Figure 4.

MAXIMAL FILTRATION FRACTION AND EFFERENT GLOMERULAR STASIS

Under extreme efferent constriction, $P_c + P_o$ will ultimately rise to a value equal to the mean systemic pressure, and consequently FF must in theory have an upper limiting value. At a mean systemic pressure of 90 mm Hg, FF cannot exceed 68 per cent for at this degree of protein concentration P_o will be 75 mm which, with $P_c = 15$ mm, will give a glomerular pressure equal to the systemic pressure, or 90 mm. Insofar as this pressure exceeds the postglomerular pressure, blood will flow through the glomeruli with the maximal filtration fraction of 68 per cent. Further efferent constriction will then reduce this blood flow without increasing the percentage of water filtered. Actually stasis will no doubt occur before the resistance of the efferent arterioles is such as to produce a maximal filtration fraction, since the abstraction of plasma water will produce a critical increase in the apparent viscosity of the blood cells, and an increase in viscosity will serve just as well to raise

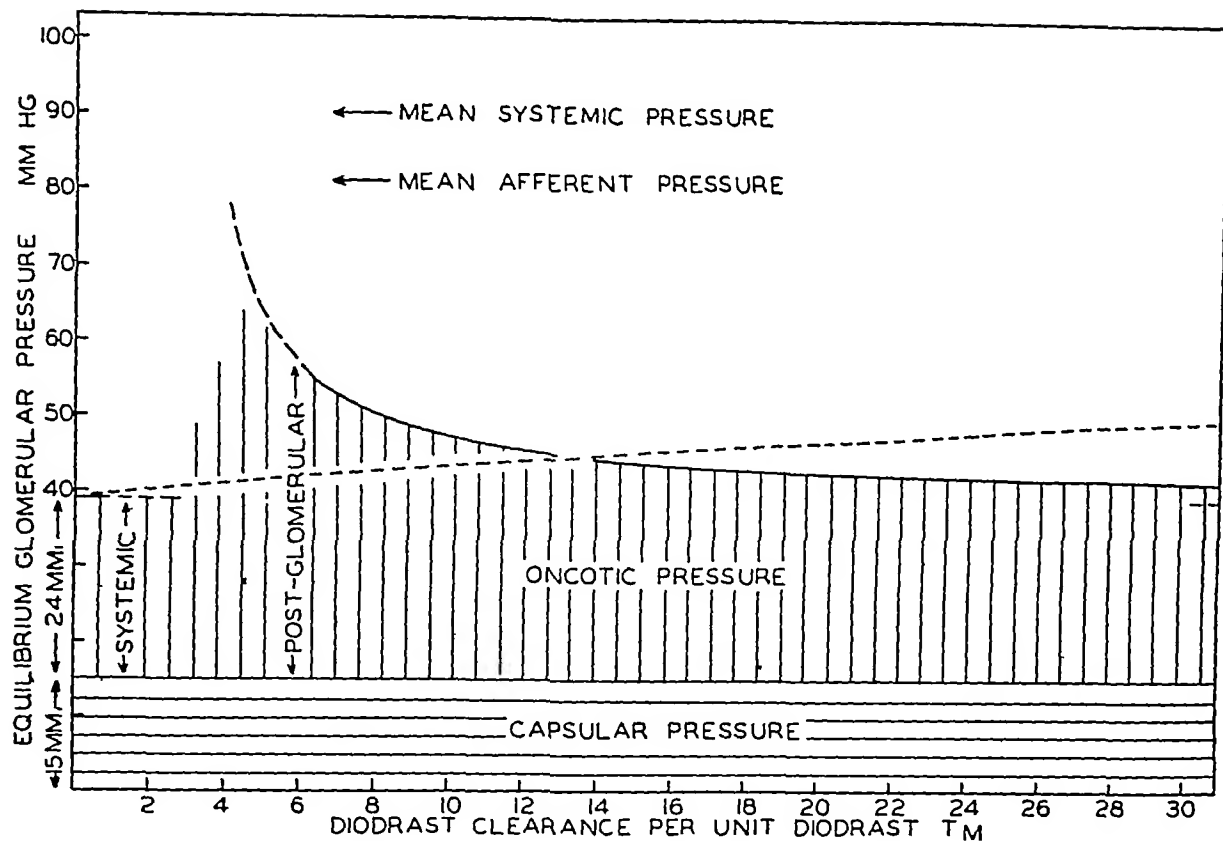


FIG 4 EQUILIBRIUM GLOMERULAR PRESSURE (P_g) CALCULATED FOR EFFERENT CONTROL (SOLID LINE) AND AFFERENT CONTROL (DOTTED LINE), ASSUMING THAT THE TONE OF THE AFFERENT OR EFFERENT ARTERIOLES, RESPECTIVELY, REMAIN FIXED AT THE NORMAL BASAL VALUES CORRESPONDING TO THE MEDIAN OF THE BASAL DATA IN FIGURE 1

P_g to the level of the systemic pressure as an increase in efferent arteriolar resistance

Whether glomerular stasis ever results in fact from efferent arteriolar constriction cannot be said at the present time. At the lowest rates of blood flow recorded here, neither the limit set on the filtration fraction by the available systemic pressure nor that set by increasing viscosity of blood has apparently been approached.

CHANGES IN GLOMERULAR PRESSURE AND BLOOD FLOW ATTRIBUTABLE TO CHANGES IN AFFERENT ARTERIOLAR TONE

(e) Where a change in blood flow results from a change in the resistance offered by the afferent arterioles (both efferent arteriolar resistance and postglomerular resistance being held constant), the effective perfusion pressure, P_E , will vary directly as the efferent blood flow, or

$$(13) \quad P_E = k_2(C_D/Tm_D - C_{IN}/Tm_D)$$

Since forward blood flow will not begin until P_g exceeds $P_c + P_o$ (as shown under (b) above),

$$(14) \quad P_g = P_c + P_o + k_2(C_D/Tm_D - C_{IN}/Tm_D)$$

This equation affords a hemodynamic description of how the efferent blood flow will vary with changes in glomerular pressure when the variable resistance is on the afferent side of the glomeruli.

Again P_g may be identified with the equilibrium glomerular pressure $P_{g'}$, at any point where this value and C_D/Tm_D and C_{IN}/Tm_D are known, the value of k_2 may be calculated, this value representing a given fixed efferent resistance. For this purpose we select the mean blood flow under basal conditions, where $C_D/Tm_D = 13.4$, $C_{IN}/Tm_D = 2.56$ and, according to equation (10), $P_g = 44.67$. From equation (14) it follows that $k_2 = 0.521$. By the introduction of this value in equation (14) and by the rearrangement of this equation, we may calculate the

values of P_G , C_{IN}/Tm_D , and FF corresponding to various values of C_D , P_B is first obtained for selected values of $C_D/Tm_D - C_{IN}/Tm_D$ by multiplying the latter by k_s , adding P_B to P_O yields $P_{O'}$, which when added to P_G yields P_G , while

$$C_D/Tm_D = \frac{C_D/Tm_D - C_{IN}/Tm_D}{P_O/P_{O'}}$$

$C_{IN}/Tm_D = C_D/Tm_D - (C_D/Tm_D - C_{IN}/Tm_D)$, and $FF = C_{IN}/C_D$. Values so calculated are shown graphically as the dotted lines in Figures 1, 2 and 4

MAXIMAL AFFERENT CONSTRICTION AND AFFERENT GLOMERULAR STASIS

If the glomerular pressure is lowered by constriction of the afferent arterioles, P_G will fall below $P_G + P_O$ and fluid will be aspirated from Bowman's capsule into the glomerular capillaries, according to equation (1). Interstitial fluid will also be aspirated into the peritubular capillaries, and the hydrostatic pressure in the latter, and therefore the pressure opposing perfusion will decrease. Insofar as blood escapes into the renal vein, it will aspirate fluid from both Bowman's capsule and interstitial space until the interstitial pressure is reduced from +15 mm to -24 mm Hg. Actually the glomerular circulation will be arrested intercurrently by viscous resistance, since the apparent viscosity of blood rises very rapidly at critical low rates of flow, a circumstance which itself obstructs perfusion of organs at low hydrostatic pressures (35). This critical increase in viscosity will probably produce glomerular stasis before an increase in afferent resistance *per se* lowers the glomerular pressure to critical values.

OBSERVED CHANGES IN THE AFFERENT ARTERIOLE TONE

It is possible to discover in the present data certain instances where changes in renal blood flow are positively correlated with changes in filtration fraction, but in all these instances the data remain within the statistical parameters which we have accepted as defining the variations in the basal condition, variations possibly attributable to differences in P_G , P_O and mean systemic pressure in different individuals or in the same individual at different times, and it

would be inconsistent with the statistical treatment to attach any contrary significance to them. The most one can say is that slight changes in afferent tone perhaps complicate what is predominantly efferent regulation. If they are to be so interpreted, these changes will be amenable to more accurate evaluation when a method is available for experimentally and reproducibly modifying the tone of the afferent arterioles.

In résumé, the observed fact that the rate of glomerular filtration tends to remain constant at varying rates of renal plasma flow must, under the premise of filtration equilibrium, necessarily be the case if the change in rate of renal blood flow is a consequence of changes in the tone of the efferent arterioles.

The effects of changes in the tone of the afferent arterioles in the face of constant efferent tone, have been described in principle. However no method of consistently altering the afferent tone is available at the present time, nor can any of the observed variations in renal blood flow in the present data be definitely attributed to changes in afferent tone.

Returning to a question raised earlier in the discussion it will be recalled that the calculations on glomerular pressure presented here are made under the premise that filtration pressure equilibrium is attained in the glomeruli. If filtration equilibrium is not attained, the glomerular pressure must exceed $P_G + P_O$ (equations 10 and 14) by whatever gradient exists between the interior and exterior of the glomerular capillaries regardless of whether afferent or efferent arteriolar changes predominate in producing the changes in the renal blood flow. It would be expected that this gradient will be roughly proportional to the rate of glomerular filtration since it represents a head of energy necessary to drive plasma water and solutes through the glomerular membranes, and that it can be closely described by adding some value which is proportional to the filtration rate (for example, $k_s C_{IN}$) to the left hand term of equations (10) and (14). But in the light of all available evidence and until definite evidence to the contrary is available, we may reasonably consider that at the efferent end of the glomerular capillary this gradient is so small as to be negligible.

It must be noted that, in view of the relationship shown in Figure 1, neither the filtration rate nor any clearance closely dependent upon it (urea, creatinine, ferrocyanide, etc) is of any value in revealing changes in renal blood flow in man under such conditions as those examined here

POSSIBLE RÔLE OF ARTERIOVENOUS ANASTOMOSES

Spanner (29) has reported the existence of anastomoses between the interlobular arterioles and veins in the human kidney, and he believes that these anastomoses permit the retrograde perfusion of the cortical capillaries at a time when the glomerular circulation is partly or wholly arrested. If such anastomoses function as postulated by Spanner, it is conceivable that their dilatation or constriction could increase or decrease the blood flow to the renal tubules, and therefore the diodrast clearance, quite apart from any change in glomerular circulation, under which circumstances the filtration rate might possibly remain constant in spite of large changes in total renal blood flow. The existence of arteriovenous anastomoses cannot be disputed in the face of Spanner's careful injection experiments, but there is no evidence at the present time that they actually do permit the retrograde perfusion of the capillaries in the intact kidney, or that their number is sufficient to increase the total blood flow substantially above that afforded by the glomerular circulation.

Springorum (30), using dogs anesthetized with pernocton, has examined the changes in arterial and renal venous pressure relative to changes in renal blood flow (thermostromuhr) under the action of adrenalin, sympatol, veritol, the carotid sinus reflexes, reflex anuria, and histamine, and has found no evidence of the functional activity of such anastomoses. We would not deny the possible importance of A-V anastomoses in shunting blood from arteries to veins under special circumstances, but we are aware of no evidence which indicates that a retrograde perfusion of the capillaries contributes significantly to the changes in blood flow described here. If retrograde perfusion does occur, it would not alter the application of the hemodynamic principles discussed above to the changes in the circulation through the glomeruli. Appropriate correction

of the total diodrast clearance for the aglomerular blood flow would increase the value of FF , and consequently of P_G , above the apparent values as now calculated, and insofar as variations in blood flow are purely tubular, would proportionally reduce the amount by which the glomerular blood flow is altered under the various experimental conditions.

The question of whether the number of "active" glomeruli is increased during hyperemia or decreased during ischemia is subordinate to the question of the existence of aglomerular channels permitting the direct perfusion of the tubules, and need not be discussed at this time.

We defer for future discussion the fact that (as judged by the urea clearance) the filtration rate in dogs maintained on low and high protein diets varies directly with renal blood flow (40). It is possible that dietary factors (in contradistinction to those examined here) act primarily upon the afferent arterioles, or it may be that in the dog the afferent arterioles play a larger rôle in the control of renal blood flow than they do in man. It should be noted, however, that the average filtration fraction is considerably higher in the dog (29.7 per cent (4)) than in man (18.9 per cent (8)), whereas the available data on the renal blood flow/cardiac output fraction indicate lower values for this ratio in the dog (18 per cent (13)) than in man (19.4/0 = 29 per cent (8)). If P_C and P_O are assumed to be the same in the two species, the meager data in the dog indicate that this animal has basally a considerably greater efferent arteriolar tone than has man.

SUMMARY

Data on the diodrast and inulin clearances (both referred to the tubular excretory mass) obtained under basal conditions and during renal ischemia and hyperemia have been analyzed with special reference to glomerular dynamics.

The concept is developed that the separation of fluid in Bowman's capsule is a process of reversible filtration comparable to the formation of interstitial fluid by the systemic capillaries, and that in the distal portion of the glomerular capillary the hydrostatic pressure and the pressure opposing it, *viz*, the sum of the plasma oncotic pressure and the capsular (renal interstitial) pressure, are equal. This equality, which

is designated as filtration equilibrium, will in principle be maintained so long as the glomerular circulation is active and Bowman's capsule contains free fluid

It is further pointed out that the perfusion of the glomeruli is opposed both by the interstitial (capsular) pressure and by the oncotic pressure of the plasma, the latter remaining effective so long as interstitial fluid exists around the peritubular capillaries and so long as this fluid is in osmotic equilibrium with the plasma at any point.

It is deduced that, where changes in renal blood flow are attributable solely to changes in the tone of the efferent arterioles the rate of glomerular filtration will be constant and independent of renal blood flow. This is in fact the case under the particular conditions of ischemia and hyperemia examined here.

Taking the plasma oncotic pressure and the capsular pressure at arbitrary values, the equilibrium glomerular pressure is calculated for various conditions of renal blood flow from the diodrast and inulin clearances.

Calculations are presented to show the effects of changes in the tone of the afferent glomerular arterioles on the renal blood flow and the rate of glomerular filtration, but it is pointed out that no method is available at the present time to alter consistently or reproducibly the tone of the afferent arterioles.

The theoretic glomerulo-dynamic factors involved in glomerular stasis, both of efferent and afferent arteriolar origin, and the significance of arteriovenous anastomoses and the retrograde perfusion of the tubules through such anastomoses, are briefly discussed.

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PROCEEDINGS OF THE THIRTY-SECOND ANNUAL MEETING OF
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION
HELD IN ATLANTIC CITY N J MAY 6, 1940

READ BEFORE THE SCIENTIFIC SESSION

PRESIDENTIAL ADDRESS

Functions and Dysfunctions of Learned Societies

BY ISAAC STARR

In times of intellectual activity men with common interests have always organized themselves into societies. I propose that we examine the *raison d'être* of such societies, ask what the aims are, and then, changing from the general to the particular ask ourselves how well we are accomplishing the aims we have in mind.

Certainly we are not a club whose aim is the entertainment of the members. We do not come together for mutual admiration and we are not concerned with perpetuating the past. We are concerned with the present and the future, and the proper aim of learned societies as I see it, is a double one first, to set a standard and second, to provide a service. What do we aim to accomplish in these directions and how successful are we in attaining our objectives?

The presidential address of our first president, Dr Meltzer was published in the *Journal of the American Medical Association* (J. A. M. A., 1909 53 508). I hope that you have read or will read it. In a fighting speech he defends the claims of clinical research against attacks emanating from two directions first, the inertia of those in authority second, the lure of what he calls the "golden calf" of practice. Though it is nowhere explicitly stated, Meltzer's ideal for this society stands out plainly it is to be composed of young men who will have the truth at any personal sacrifice. May this remain our ideal still!

But times have greatly changed since 1909. The interest in research has increased until it is now a matter for newspaper headlines and the only criticism that could be made of those in authority who are so often among the founders of our society is that in certain places there seems to be some lack of discrimination between the search for truth and the writing of papers. But today all pay lip service to research if they pay no more. So we find ourselves in a situation quite different from that existing when our society was founded and it is time to reconsider our aims.

The service we aim to supply may be considered under three headings. Let us take first that rendered by the *Journal of Clinical Investigation*. To those of us who are considered to be clinicians by physiologists, biochemists, and immunologists and considered to be physiologists, biochemists, or immunologists by most clinicians the existence of this journal has been a godsend. Without any particular effort to direct the subject matter sub-

mitted, it has become the locus of the papers concerned with clinical investigations made by quantitative methods and so it is in the forefront of that change from descriptive and qualitative methods to the more exact quantitative measurements, a change which has made possible the great advances of physics and chemistry and which may well produce as great a revolution in medical practice. Let us pause to pay tribute to the Rockefeller Foundation whose financial support made the starting of our *Journal* possible, to the medical clinics, and to the Chemical Foundation which assisted it at a time of need, and especially to the editors who entirely without financial recompense, have given so much time and effort to making the *Journal* what it is.

Our second method of service is in these meetings. I find that any attempt to read the medical literature systematically is overwhelming. I am counting more and more on meetings such as this to keep me on the firing line. But I sometimes wonder whether greater service might not be possible. Our programs have contained very little of what may be called synthetic thinking. It is true that 100 years ago, and more, medical speculation was almost wholly fanciful. From this we were rescued by the great pathological school with its insistence on demonstrated facts, and as a result the promulgations of theories became not quite *comme il faut*. But, on the other hand, facts are not of equal importance. In every investigation there is a period when one must decide which facts are worth discovering. To do this logically one must picture to himself what the situation is. In this connection I want to point out a difference between clinicians and other scientists which is sometimes a cause of misunderstanding. Chemists, physiologists, and the like, working in pure science, are under no pressure to make up their minds. If their grasp of any situation does not please them, they can wait any length of time before committing themselves. But a clinician, confronted with a patient, must come to a decision, and to act intelligently he must create a picture of the situation, even though the evidence on which to base it is scanty indeed. Hence, clinical theories are, by the very nature of things, less firmly based than those in the branches of pure science. But they are not less important and, in my opinion our program would be improved by studying the methods of the great English school of physi-

ology whose members have never been afraid to attempt to synthesize the facts into simplifying theories

It is true that some persons, like imaginative children, seem quite unable to distinguish the facts from the figments of their imagination, but this is no excuse for failing to try to synthesize the facts we have. Scientists who have never made an observation or conducted an experiment may contribute signally to our understanding of nature. If one were only clever enough what might not be gleaned from the multitude of observations and experiments in the medical literature and from the long stacks of hospital records now gathering dust? We are spending much time, effort, and money in our clinics accumulating records, apparently in the pious hope that some day somebody is going to synthesize something useful from the multitude of data they contain.

Our third method of giving service is less tangible, but I believe that it is very important. It consists of the advantage that comes with a wide acquaintance among scientific investigators. The ideal doctor, like the philosopher, should have at his command the sum total of all knowledge and any idea that one man can cover the field of modern knowledge applicable to medicine is preposterous. We must cooperate. I hope that membership in this society makes it easier for young investigators to ask questions and obtain criticism from those whose experience and effort are in a different direction from

I am convinced that a few questions asked of just the right people will usually secure a nearer approximation to the truth than can be discovered in many months of patient search of the literature. I hope that personal contact at our annual meetings may make such things easier

If investigators would think of their confreres not as rivals but as friends cooperating against common difficulties, much would be gained for science in America. It is my hope that this society will bring about friendships of this sort and so avoid the silly acrimonious controversies which have so plagued some branches of science.

I think few people have any doubts about the value of the services rendered by this and other learned societies, but the difficulties begin when they start to exercise their other function, that of setting a standard.

We set standards when we select members for our society, and here our standards have changed. When this society was four years old the secretary sent out a circular letter urging the members to nominate friends who might be interested. Now the waiting list far exceeds the present constitutional limit of membership! In the earlier days interest in research was deemed sufficient. Now we demand tangible evidence of accomplishment. The descriptive essays which have played so large a part in medical literature are not good enough for us now.

The tremendous growth of research interest in America has greatly increased the number of available candidates and from those your council must try to select the best on the basis of the candidate's publications, his academic rank, and the letters from members about him. As is

inevitable, institutional pride and personal friendship color the recommendations so that the council has a hard time deciding. One problem is particularly bothersome. Candidate A has worked in a laboratory known to be highly productive, and its resources have been at his command. His production is superior to that of B, but B has been working without either scientific or financial support. Which is the better man? Time will tell but until it does I do not think the question can be answered with confidence, and when I admit this I admit that mistakes in the application of our standard are inevitable.

A second method of setting our standard is in the selection of papers for the program. This duty of the president must be performed by inspection of abstracts in a very short period of time. Again mistakes are inevitable. The selection is not always on the basis of intrinsic merit, general interest, timeliness, and the ability of the author to present his work clearly, *must be considered*. But due to the energy and cooperativeness of the members who annually submit about four times the number of titles which can be accommodated, I do not believe that there is another medical program of comparable size which, year in and year out, contains so much of real interest.

Our third method of maintaining a standard is by the selection of papers for our Journal. With this I personally have had *no experience*, but several facts should be brought to the attention of the members. Like the other scientific journals owned by learned societies, the Journal of Clinical Investigation was not born because of a widespread demand on the part of the membership. In its early days it was proper and inevitable that control should reside with the group who initiated it and who were in a position to finance it. But now, although our Journal is self supporting, the membership as a whole has neither taken the interest it should nor exerted the authority it possesses in the Journal's affairs. I suppose that this indicates complete satisfaction with things as they are. If it does not, it is the fault of the membership itself. Helpful criticism by members has been conspicuous by its absence and it might do much to strengthen the Journal.

During the foregoing discussion of the functions of learned societies, perhaps my next point has already occurred to you. It is that the two chief functions of learned societies are, in a measure, in conflict. Thus there are some who think that the membership should be enlarged and the program expanded. Their aim is to improve the service given by the society. This is opposed by others who point out that such a change would lower our standard of excellence.

Too much emphasis on standards is a cause of decay, often it is a psychological defense mechanism set up by persons no longer productive. The organizations which become more and more exclusive tend to die of dry rot. Mistakes made in the enforcement of the standard make them ludicrous.

I often reflect on our own shortcomings. The young

Pasteur, if nominated to this society would probably have been turned down because he was a chemist the young James Mackenzie because he was not connected with a medical school.

Nevertheless, while undue insistence on standards causes difficulties, abandoning them too far in the interest of service brings troubles of another sort in its train. For then the door is opened for the man whose real aim is personal advertisement rather than the search for truth. Uncensored programs are likely to be too long. Societies with low entrance requirements become so large that friendship between the members becomes impossible and the personal service on which I lay so much value is non-existent.

Somewhere, therefore, between the aim of setting a standard and the aim of giving a service, a compromise must be made. It is not for me as your presiding officer to dictate any line of conduct but I am going to suggest that the members, in attacking this and the other problems which confront learned societies, keep two principles in mind.

To illustrate the first I have, under the urge of a sense of duty as your representative, constructed a model of the society's activities.* Here I have a representation of our society radiating its beneficent influence in almost every direction. Now this timepiece is an integral part of the model for it indicates that the influence of our society extends and will extend throughout time. But the timepiece has another purpose which is important, for by means of it I am entitled to claim that our model is a four dimensional one and that it is therefore one (dimension) up on the model produced by my friend the past president of the Association of American Physicians, at the last meeting of that distinguished body (DuBois, E. F., *Tr. A. Am. Physicians* 1939 54 1).

But, in spite of all this, I stated that my model was designed to illustrate a point in my argument. The point is this in dealing with learned societies, let us preserve our sense of humor. In some of the letters received by the secretary a sense of humor has seemed strangely lacking. The errors of today if they be errors can and must be corrected tomorrow.

As a final point I suggest a larger use of the ordinary processes of democracy in the solution of our problems. There has been a tendency in American learned societies to let the officers make all the decisions behind closed doors. The attendance at business meetings has become smaller and smaller and the proceedings more and more routine. In my opinion this is an unhealthy tendency for I conceive that it is the duty of the officers to keep the members informed of the problems which concern them and that the members have an obligation to assist in making the necessary decisions. If our society is to

be made maximally effective, it must truly represent the best thought of the younger minds in medical investigation. The decision concerning its policies rests with you. And these decisions are important because there is no reasonable doubt that nowhere in this war-torn world is there a group of young men whose opportunities for the advancement of medical research can be compared with those which have been granted to you.

The Chemical Properties of Scarlet Fever Toxin. By E. S. GUZMAN BARRON and (by invitation) GEORGE F. DICK and CARL M. LYMAN Chicago, Ill.

The chemical properties of the scarlet fever toxin purified by Dick and Boor were studied by using skin reactions in human subjects to measure the activity of the toxin.

The toxin, as shown by other investigators, is very resistant to heat, for it can be heated for 1 hour up to 90° C. and for 45 minutes up to 100° C. with no loss of activity. Nor was there any loss of activity when it was subjected to the action of trypsin and pepsin for 24 hours. The toxin is also resistant to pH changes, for it remained active when kept for 24 hours at 25° C. from pH values of 1.08 to 11.18. It is resistant to the action of oxidizing agents such as H_2O_2 , copper and oxidized glutathione, and to that of reducing agents such as cysteine, glutathione, $Na_2S_2O_4$, and H_2 activated with Pt. black. Iodine and porphyrin destroyed it by destroying free amino groups. Neither sulfanilamide nor its oxidation product obtained by irradiation with ultraviolet light had any action on the toxin. The isoelectric point determined with Theorell's cataphoresis cell at 3° C. was at pH 5.48. At pH 4.5 the toxin migrates to the cathode and the protein impurity to the anode branch of the cell, permitting further purification. The toxin is destroyed by 30 minutes treatment with ketene and nitrous acid, showing that the presence of amino groups is essential for activity. On ultrafiltration through graded collodion membranes, the toxin filtered through membranes of a porosity that did not let cytochrome C pass through (molecular weight of cytochrome C, 13,000) on the other hand membranes that let clupeine through (molecular weight about 2500) did not let the toxin through.

It may be concluded that scarlet fever toxin is a polypeptide with a molecular weight between 13,000 and 4,000 its activity being determined by the presence of free amino groups.

The Significance of Antibodies Against Epidemic Influenza Virus. By E. R. RICKARD and EDWIN H. LYN NETTE (by invitation) and FRANK L. HORSFALL, JR. New York, N. Y.

The respiratory disease experience of a representative suburban population of 800 individuals has been studied for 2 years. Blood specimens were obtained from all individuals in this group in 1938 and again in 1939. The titer of antibodies against the PR8 strain of epidemic influenza virus was determined on all these sera both by means of the neutralization and complement

* At this point the president drew forth a curious looking object which at a distance resembled an orange thrust through with a multitude of long steel knitting needles that radiated from it in all directions. This he placed beside a clock.

and was found to differ markedly from one individual to another

In the first 3 months of 1939 a small epidemic of influenza occurred in this population. The incidence of clinical cases, in which the diagnosis was confirmed by the isolation of influenza virus or by a significant increase in antibodies, was 10 times greater among persons who possessed low neutralization titers than among those whose titers were high.

With the sera of 600 individuals who did not have influenza during the epidemic and who presented no evidence of subclinical infection, it was found that individual antibody titers, irrespective of their initial level, remained constant for a period of 1 year

Rat Bite Fever With Arthritis Due to Streptobacillus Moniliformis By THOMAS MCPHERSON BROWN and JOHN C. NUNEMAKER (introduced by Perrin H. Long), Baltimore, Md.

A 49-year-old farmer was bitten by a rat and 18 days later was admitted to the Johns Hopkins Hospital because of fever and arthritis. The streptobacillus moniliformis was isolated from the joint fluid and blood. Severe arthritis localized in the left knee, with destructive changes by x-ray, and also marked laryngeal changes were the chief clinical features. No definite improvement was noted from aspirin or sulfanilamide, there was apparent benefit with gold therapy. Streptobacillus moniliformis has been agglutinated in high titer by patient's serum during a four months' period, also by serum from immunized rabbits.

L_1 pleuropneumonia-like organism of Klieneberger has been isolated from cultures of the streptobacillus moniliformis. L_1 colonies have remained constant on solid media, but have always reverted to streptobacillus form when transferred to liquid media, and then back to solid media.

Migratory polyarthritis has been observed in mice injected both intravenously and intraperitoneally with the streptobacillus moniliformis. The experimental arthritis has been illustrated by photographs, x-rays and sections, also by diagrammatic drawings of joints involved, indicating degree of involvement and migratory character of the arthritis. Gold prevents infection of mice with this strain of streptobacillus moniliformis. Sulfanilamide and sulfapyridine produce no therapeutic effect.

The Occurrence and Significance of a Factor Which Annuls the Bacteriostatic Action of Sulfonamide Compounds By C. M. MACLEOD and G. S. MIRICK (introduced by O. T. Avery), New York, N. Y.

The presence of a substance in peptone which greatly diminishes the bacteriostatic action of sulfonamide compounds *in vitro* was observed first by Lockwood. The occurrence of this material in the peptones used in the preparation of the usual bacteriological media has made comparative bacteriostatic tests difficult to interpret, since different lots of media prepared under apparently identical conditions may contain different amounts of the

sulfonamide inhibitor. However, the presence of this substance is not restricted to peptones since it has now been found to exist in certain body tissues. Increased amounts of the inhibiting factor are associated with the occurrence of autolysis in tissues and exudates. Thus, fresh muscle contains only a small amount, but if autolysis takes place a great increase in inhibitor occurs. Purulent exudates obtained from patients with staphylococcal, pneumococcal and streptococcal infections, as well as guinea pig liver containing caseous tuberculous lesions, yield large amounts. Fresh spleen is rich in the substance, but none has been demonstrated in fresh liver. This inhibitor is of importance, therefore, not only with respect to *in vitro* bacteriostatic tests but also in relation to the lack of bacteriostatic action of the sulfonamide compounds observed clinically in the presence of purulent lesions.

Sulfapyridine and Vomiting: An Experimental Study of the Mechanism in Dogs By JOSEPH F. SADUSE, JR. and JOHN W. HIRSHFELD, with technical assistance of ANNE SEYMOUR (introduced by Francis G. Blake), New Haven, Conn.

Vomiting in dogs following the administration of sodium sulfapyridine intravenously is ordinarily produced when the blood level of sulfapyridine reaches 20 to 30 mgm. per cent. This vomiting level is not materially changed if, preceding the intravenous injection of sodium sulfapyridine, the installation into the stomach of as high as a one per cent suspension of sulfapyridine is done.

Experiments carried out following total gastrectomy, and even total removal of the gastro-intestinal tract, revealed no change in the blood level at which vomiting occurred. A preliminary experiment concerning the local effect of sulfapyridine and apomorphine upon the vomiting center in the medulla would seem to indicate that the vomiting is not primarily central in origin.

With the data noted above, it would appear that the mechanism of vomiting in dogs following sulfapyridine administration is dependent on a reflex stimulation of the vomiting center from some organ other than the gastro-intestinal tract.

The Clinical Significance of Gastro-Intestinal Pressure Changes By W. O. ABBOTT and (by invitation) H. K. HARTLINE, J. P. HERVEY, F. J. INGELFINGER, A. J. RAWSON and L. ZETZEL, Philadelphia, Pa.

The movement of contents within the gastro-intestinal tract occurs in response to changes in the internal pressure of the gut just as the movement of blood is governed by the relation of arterial to capillary and venous pressures. A new method has been devised which makes it possible to record the pressures simultaneously at several selected points along the alimentary tract at two-second intervals. Normal movements of the intestinal contents are observed by the fluoroscope while the pressures are being recorded. Thus the gradients of pressure in the intestinal canal and the movements engen-

dered by them may be observed directly in normal subjects and in patients suffering from digestive disorders. The records obtained are interpreted in the light of normal and abnormal gastro-intestinal reactions, and the diagnostic value of such data is presented.

An Investigation of the Mechanism of Experimental Nephritis Produced in Rabbits by the Use of Anti Nephrotoxic Duck Serum. By CALVIN F KAY (by invitation) (introduced by WARFIELD T LONGCOPE) Baltimore, Md.

A study was first made of the relationship between the appearance of antibodies to duck serum and the appearance of nephritis in rabbits injected with nephrotoxic serum. It was found possible by appropriate means either to shorten or to lengthen the interval between the onset of nephritis and the appearance of antibodies. The duration of this incubation period was found to be related to the development of the antibodies to duck serum. Evidence is presented to indicate that antibody formation by the rabbit is essential for the development of the nephritis. An hypothesis is presented to explain the mode of action of the nephrotoxic serum.

The Use of Radioactive Sodium as a Measure of the Volume of the Extracellular Fluids of the Body By NOLAN L. KALTREIDER, GEORGE R. MENNELLY, JAMES R. ALLEN, STANLEY N. VANVOORHIS and VINCENT F. DOWNING (introduced by Wm. S. McCann) Rochester N Y

Radioactive sodium was produced by the bombardment of sodium chloride with deuterons. Its half life is 14.8 hours. The quantity of radio sodium present in a sample was determined by a measurement of its ray activity using a Geiger Muller counter. By means of this artificially produced radioactive substance, the authors were able to measure the amount of fluid available for the dilution of sodium. Simultaneously for comparative purposes the volume of fluid through which thiocyanate is distributed was estimated after intravenous injection of sodium thiocyanate. The plasma volume was measured concurrently by the dye (T-1824) method.

In normal subjects it is apparent that the attainment of diffusion equilibrium for radio sodium and thiocyanate between plasma and the interstitial fluid was reached in 3 hours. The average value for the "sodium space" of 8 normal subjects was 19.55 liters, or 26.5 per cent of body weight, with extreme values of 23 and 29 per cent. The amount of available fluid appeared to be more closely related to the body surface area than to the weight.

In order to attain equilibrium of radio sodium between plasma and various transudates in patients with congestive heart disease, 6 to 12 hours were required. During congestive failure both the plasma and interstitial volumes were increased and, as improvement took place, both volumes diminished. During recovery the decrease in the volume of extracellular fluid paralleled the loss of body weight. Both in normal subjects and in patients who showed an accumulation of fluid, the volume meas-

ured by radio sodium was higher than that found by thiocyanate. In contrast to thiocyanate, radio sodium diffused into the subarachnoid space.

The Action of Adrenalin on the Ischemic Kidney and the Response of Hypertension to Tyrosinase By HENRY A. SCHROEDER (by invitation) and ALFRED E. COHN New York, N Y

Blood pressure in anesthetized dogs was recorded by Hamilton's manometer and renal blood flow by a thermistor-muhr. Records were made during various degrees of constriction of the renal artery the animals being watched for several hours. It was found that small doses of adrenalin given intravenously resulted in prolonged and sustained falls of blood flow in the affected kidney when the renal artery was constricted, producing no demonstrable effect in the other kidney. One-hundredth of the dose of adrenalin necessary for transient reduction in blood flow in the normal kidney resulted in marked vasoconstriction for 5 to 30 minutes when the kidney was ischemic. This effect was noticed with amounts as low 0.05 μ per kgm. of dog. The greatest response occurred when the renal artery was constricted to a point at which intrarenal vasodilatation described previously, was maximal.

In eight of eleven dogs systemic blood pressure became significantly elevated for the duration of the experiment (3 to 6 hours) after constriction of the renal artery and further reduction of renal blood flow by small doses of adrenalin, while constriction of the artery alone resulted in little change in blood pressure. The intravenous injection of tyrosinase, a pure phenolic oxidase prepared by and obtained from Prof. J. M. Nelson, lowered blood pressure raised by this method. Likewise, tyrosinase, when given intravenously to fifteen hypertensive rats with unilateral renal affections, lowered blood pressure consistently this effect appearing 5 to 15 minutes after injection and lasting as long as the rats were followed (in five and male—1, 5, 14, and 17 days). The injection of this enzyme into twenty normal rats gave inconsistent results. Intravenous and intramuscular injections in four hypertensive dogs lowered systolic blood pressure for 3 to 48 hours, there being little effect beyond an immediate one in the normal. The effect on diastolic pressure was of lesser degree. It appears possible that these results are produced by the alteration of a phenolic configuration in some substance.

The Role of the Central Nervous System in Renal Hypertension. By WILLIAM DOCK, San Francisco Cal.

When one pits the central nervous system of rabbits hypertensive because of infusion of epinephrine or renin, there is little or no fall in arterial pressure until the effect of the drug wears off. If rabbits with renal hypertension are pithe, the pressure falls swiftly to the same low levels as it does in pithe controls. The pithe rabbits, control or hypertensive, respond to epinephrine with out any rise in venous pressure but with a rapid rise in arterial pressure raising venous pressure by salt or

The Excretion of Cortin under Conditions of Damage

By PAUL G WEIL* (by invitation) and J S L BROWNE, Montreal, Canada.

Studies on the cortin-like action of extracts of urine have been reported by Perla and Marmorston in 1931, by Grollman and Firor in 1933 and by Harrop and Thorn in 1937. In 1938 Anderson, Haymaker and Joseph showed that the blood and urine of patients with Cushing's syndrome contained cortin. In 1938 Selye and Schenker devised a method for the assay of cortin, using the immature adrenalectomized rat exposed to cold. The method is specific, rapid and one hundred times more sensitive than the ordinary survival tests.

The urine is extracted with ethylene dichloride and the residue remaining after distillation of the solvent is taken up in water and administered to the test animals by stomach tube. Using this sensitive test it has been shown that certain individuals excrete cortin in detectable amounts. Cortin can be recovered from the urine of patients after injection of either adrenal cortical extract or desoxycorticosterone acetate. No cortin was found in the urine of twelve normal individuals of both sexes of various age periods. Cortin was found in the urine of seven patients with the adrenogenital syndrome (Cushing's syndrome one, hirsutism six), of four out of six cases of hypertension, of ten patients with acute and chronic infection including one case of pneumonia, one of subacute bacterial endocarditis, two of otitis media, two of chronic osteomyelitis, one of pneumonitis, two of influenza, one of tetanus, two cases following burns, and ten cases after surgical operations. One of the patients with pneumonia excreted relatively large amounts of cortin during the height of the disease. As his condition improved cortin disappeared from the urine. Certain urines, including those of patients receiving large amounts of sulphamidamide and sulphapyridine have been found to be toxic. The action of toxic substances, such as the excretion products of the above mentioned compounds, in obscuring the cortin action of the urine is thought to be responsible for the failure to demonstrate cortin in the urine of six cases of pneumonia and four other cases of infection. The possibility of the presence of toxic substances in urine extracts must always be considered. The absence of detectable cortin activity in normal urine may represent a balance between secretion by the adrenal cortex and its utilization by the body on the assumption that cortical substances are inactivated in the course of their utilization. According to this view the appearance of cortin in the urine may represent the difference between secretion and utilization. The cortin present in the urine may not therefore reflect quantitatively the degree of increased secretion of the adrenal cortex. A lowering of renal threshold for cortin under conditions of damage cannot be excluded at present but seems unlikely.

The increased secretory activity of the adrenal medulla as part of the defense mechanism of the organism was

pointed out by Cannon. An increased excretion of cortin under conditions of damage such as infections and trauma is considered to be a manifestation of the response of the organism to a damaging stimulus by an increased secretory activity of the adrenal cortex. It has been shown that the adrenal cortex of the laboratory animal hypertrophies after the animal has been exposed to a noxious stimulus. It is suggested that the increased secretion of the adrenal cortical hormone forms part of the protective mechanism of the body against damage.

Studies on the Role of the Adrenal Cortex in Carbohydrate Metabolism

By GEORGE W THORN and (by invitation) GEORGE F KOEFF and ROGER A. LEWIS, Baltimore, Md.

The following disturbances in carbohydrate metabolism were observed in a majority of a group of 25 patients with classical signs and symptoms of Addison's disease.

1 A rise, greater than normal, in the non-protein respiratory quotient following intravenous glucose administration.

2 A marked inclination to develop hypoglycemia 3 to 4 hours after the administration of glucose orally or intravenously.

3 A decreased glycemic response to a standard dose of epinephrine.

4 An inability to maintain a normal glycemic level during a prolonged fast.

The persistence of a disturbance in carbohydrate metabolism after treatment has restored electrolyte balance, plasma volume and blood pressure to normal suggests that the adrenal cortex has a direct effect on carbohydrate metabolism. It is evident that, whereas the correction of the underlying disturbance in carbohydrate metabolism is essential for complete restoration to normal function, the carbohydrate-regulating effect of the adrenal cortex is not necessary for the maintenance of life in the absence of complications.

"Diabetes Insipidus" Induced in Normal Animals by Desoxycorticosterone Acetate

By CHARLES RAGAN, JOSEPH W FERREBEE, P PHYFE (by invitation) and DANA W ATCHLEY and ROBERT F LOEB, New York, N Y

In the course of experiments on the effects of desoxycorticosterone acetate in normal animals, we have observed the development of "diabetes insipidus". The severity of the diabetes insipidus has varied considerably in different animals and is definitely augmented by the administration of sodium chloride. A recent study of one of the dogs with "diabetes insipidus" is best demonstrated by means of the accompanying chart. It will be observed that the fluid intake at the beginning of the chart was about 6000 cc. a day. Fluid output is not charted because measurement of urine volume and weight indicated that the dog was in fluid balance. It will be noted that large doses of pitressin had some effect in that they raised the specific gravity and lowered the water

*Aided by a grant from the Banting Research Foundation.

intake. Following the withdrawal of pitressin, fluid intake rose to a level higher than that before this substance was administered and the specific gravity of the urine fell. When fluid intake was restricted to 2500 cc. for a day the specific gravity again rose as in the case of pitressin administration. One difference, however is to be observed, namely that the concentration of sodium in the serum rose sharply with fluid restriction, whereas no change occurred at the time pitressin was given. When desoxycorticosterone was discontinued the fluid intake dropped in seven days to 1500 cc., despite the fact that the daily ration of 8.5 grams of sodium chloride was continued. With this decrease in fluid intake, there was a striking increase in urine specific gravity but no increase in blood sodium level. In this respect the administration of pitressin was qualitatively like that observed upon the withdrawal of excess cortical hormone.

With the readministration of desoxycorticosterone the water intake again rose and the specific gravity of the urine fell, although not to a degree as extreme as on previous occasions. Restriction of fluid again was accompanied by a sharp increase in specific gravity and also in the sodium content of the serum. From these observations it would appear as has been suggested by Silvette and Britton in their work with cortical extract, that pitressin and desoxycorticosterone are antagonistic in their action. It is tempting to speculate that desoxycorticosterone increases the reabsorption of sodium by the renal tubules and that this action is opposed by posterior pituitary substance. Studies on rats and further studies on dogs are in progress at the present time.

Effects of Synthetic and Natural Estrogens on Blood Liver and Bone Marrow By CYRIL M. MACBRYNE, DANTR CASTRODALE, ELSON B. HELWIG, OLGA BIERBAUM and JOHN S. POE (introduced by David P. Barr) St. Louis, Mo.

The advent of synthetic estrogens into therapy has raised the question whether they may be harmful. About 15 per cent of our patients treated with stilbestrol have experienced nausea and a few have vomited. Early animal experiments have suggested the possibility of changes in blood and liver resulting from stilbestrol. We have attacked this problem by simultaneous study of patients and experimental animals (dogs and rats). Fourteen patients receiving from 10 to 5.0 mgm. stilbestrol daily have shown no deviation from normal in repeated liver function studies or blood examinations.

Dogs receiving from 10 mgm. to 100 mgm. stilbestrol daily (from equivalent to 100 times the maximum human therapeutic dose) have exhibited changes ranging from simple anemia to thrombocytopenic purpura and severe anemia with a marked hypoplasia of the bone marrow. Areas of fatty degeneration of the liver are observed.

We have, however, demonstrated similar changes with comparable doses of estradiol, so that at present it seems doubtful whether the synthetic estrogens are any more toxic than those obtained from natural sources. Thrombocytopenia and anemia also resulted in rats treated with

comparable doses of stilbestrol and estradiol at dosage levels corresponding to 5 to 10 times the maximum therapeutic dose.

Radiocardiograms—the Electrical Impedance Changes of the Heart in Relation to Electrocardiograms and Heart Sounds By JAN NYDØER, SAM BAGNO, A. BARNETT and R. H. HALSBY (introduced by H. O. Mosenthal) New York N. Y.

Using a vacuum tube method of broadcasting and detection at high radio frequency high accuracy has been attained in reproduction of a new type of cardiogram which may be called the radiocardiogram. The significance of these curves, although still uncertain, shows a very close relation to the mechanical volume changes of the heart. Marked variations in curve contour and stroke amplitude are produced by valvular lesions and arrhythmias such as extrasystoles, sinus arrhythmias and auricular fibrillation. These tracings have a quantitative significance and are evaluated in terms of minute-volume cardiac output. Thus comparative values of 4000 cc. per ventricle in humans, 150 cc. per ventricle in anesthetized cats and 20 cc. per ventricle in frogs have been found as the cardiac output under given conditions. Comparative changes are noted following exercise, injection of metrazol, bleeding and during decompensation and recovery. Simultaneous electrocardiograms or heart sounds identify the relative changes in the impedance curves during the cardiac cycle.

"Radiocardiograms are altered especially in order to observe the rate of change of electrical impedance. In other words, the probable velocity with which the blood changes during the cardiac stroke is registered. This type of curve is a differentiated cardiac electrical impedance and may be named "a differentiated radiocardiogram." The auricular and ventricular input is usually registered as a sharp valley or peak, respectively.

Equations defining the quantitative aspect of cardiac electrical impedance have been checked mathematically and practically but are withheld from this abstract.

Objective Measurement of Relative Intracranial Blood Flow in Man By EUGENE B. FERRIS, JR. and (by invitation) MILTON ROSENBAUM, EPHRAIM ROSEMAN and NATHANIEL BROWER, Cincinnati O.

Utilizing the principle of the venous occlusion plethysmograph an objective method for comparative measurement of intracranial blood flow is described. The index of blood flow is obtained by recording the maximum rate of cerebrospinal fluid displacement (through a lumbar puncture needle 2 mm. inside diameter) during sudden compression of the neck veins. The outflow needle is connected to a reservoir partly filled with Ringer's solution, which in turn is attached to a Brodie bellows for recording volume changes. By adjusting the level of fluid in the reservoir intracranial pressure and volume can be maintained at any desired level. When a suitable outflow record is obtained, pressure on the neck is re-

leased and the displaced cerebrospinal fluid returns from the reservoir to the subarachnoid space.

By controlling sources of error, such as blockage of the outflow needle, leakage of cerebrospinal fluid, and ineffectiveness of the neck cuff in transmitting pressure to the deep neck veins, maximum outflow rates of similar magnitude have been obtained in fourteen control subjects and in four subjects on whom the experiment was repeated one to two times. The variation in control outflow rates obtained consecutively during each experiment was slight.

The transient effect of hyperventilation, CO₂ inhalation, and temporary elevation of intracranial pressure is similar to that obtained by other methods. Nicotinic acid causes a significant increase in flow. The cerebral blood flow has been significantly diminished in paresis and in cortical atrophy.

The Effects of Patency of the Ductus Arteriosus on the Circulation By C SIDNEY BURWELL and (by invitation) EUGENE C EPPINGER and ROBERT E GROSS, Boston, Mass.

Gross' operation (J. A. M. A., 1939, 112, 729) for ligation of the patent ductus arteriosus has permitted the application of the direct method of Fick to the measurement of pulmonary and peripheral blood-flow in these patients. Samples of blood were obtained from the pulmonary artery and from the aorta before and after the ligation of the ductus. Samples were drawn also from the pulmonary artery within fifteen to twenty seconds after occlusion of the ductus to establish the oxygen content of mixed venous blood leaving the periphery when the ductus was open. To test the validity of the method similar observations were made in dogs with the subclavian artery opening into the pulmonary artery.

The studies in patients showed that in the presence of a patent ductus arteriosus observed during operation

1 Blood from the aorta enters the pulmonary artery via the ductus

2 The amount of blood flowing through this shunt is from 45 to 75 per cent of the left ventricle output.

3 The total blood-flow through the pulmonary artery in these patients is between 8 and 19 liters per minute.

4 The blood-flow to the periphery is between 3 and 6 liters

5 Therefore, the output of the left ventricle is 2 to 4 times that of the right, presumably because blood from two sources enters the left ventricle.

Objective Evidence of the Efficacy of Certain Drugs in the Treatment of Angina Pectoris By JOSEPH E. F. RISEMAN and (by invitation) A. S. FREEDBERG and ERWIN SPIEGEL, Boston, Mass.

Electrocardiograms taken continuously during the induction of attacks of angina pectoris by exertion show that a depression of ST 4 occurs during exertion and reaches a maximum when the patient is forced by precordial pain to stop work. This depression varied between 1 and 4 mm. in twenty-six patients studied, always

occurred during attacks induced by exertion or anoxemia and was constant for each patient.

The effect of medication (nitroglycerine, octyl nitrite, theobromine sodium acetate and quinidine sulfate) on the electrocardiogram during work and on the amount of work which could be done under standardized conditions before inducing pain was studied in thirteen patients. When lactose or sodium bicarbonate (grains 5) was given four times daily for one week with an additional dose one to three hours before the test, the amount of work which could be performed and the electrocardiographic results were similar to those obtained when no medication was given. When, however, theobromine sodium acetate (grains 7½) was given, ten of the patients were able to do more work without developing pain and exercise was accomplished without inducing the characteristic anoxic electrocardiographic changes. The same results were obtained in eight patients receiving quinidine sulfate (grains 5), and in eleven who exercised two minutes after taking nitroglycerine (1 to 200 grains). Following the administration of nitroglycerine or octyl nitrite, patients were able to do much more work than after other drugs and the electrocardiographic changes were not induced by a 100 per cent increase in work, the duration of this beneficial effect, however, was not as prolonged as after theobromine sodium acetate or quinidine sulfate.

These results, which provide evidence that theobromine sodium acetate and quinidine sulfate, as well as nitroglycerine and octyl nitrite, are of value in the treatment of angina pectoris, are contrary to the clinical opinions expressed in the literature (Evans and Hoyle, Gold, Kwit, and Otto) that no drug is of value in the treatment of angina. The results are in accord with the theory that the administration of nitroglycerine or the purines results in an improved blood flow through the coronary tree.

Observations on the Effects of Intravenous Injection of Histamine in Cases of Ménière's Syndrome By BAYARD T. HORTON and (by invitation) GUSTAVUS A. PETERS and C. HUNTER SHELLEN, Rochester, Minn.

Twenty-seven subjects, many of whom were totally incapacitated, were treated by the intravenous injection of 2.75 mgm. of histamine diphosphate dissolved in 250 cc of physiologic saline solution. The vertigo promptly disappeared, tinnitus was relieved in a small per cent of the cases and the patients were able to return to work.

Special studies were made to determine the bodily responses to the administration of the drug. These studies included erythrocyte counts, leukocyte counts, differential counts, and determinations of the basal metabolic rates, the blood sugar, the concentration of individual fats and chlorides in the plasma, the concentration of potassium and protein in the serum, the albumin-globulin ratio, and the carbon dioxide combining power of the plasma, respectively, before and after the intravenous injection of histamine. Electrocardiograms were made, the surface temperatures were determined and samples

of gastric contents were analyzed, respectively before, during and after administration of the drug. All subjects were in a basal state, under controlled environmental conditions. The most striking observations were the marked rise in many instances, in the concentrations of the fatty acids in the plasma, in cases where the fatty acids were normal, the potassium in the serum and the blood sugar the decrease of the concentration of cholesterol in the plasma and the decrease in the hematocrit values. Inversion of the T wave in lead III in the electrocardiogram and occasional extrasystoles were observed. The response of the gastric acids was always maximal even though histamine was frequently administered so slowly that it did not accelerate the heart rate. The basal metabolism was not affected during slow rates of administration but was increased during rapid administration of the drug. No untoward effects were obtained in approximately 400 intravenous injections.

Infectious Feline Agranulocytosis. Kodachrome Moving Picture Demonstration of the Circulation of the Omentum as Affected by Serum from a Cat With This Disease By JOHN S. LAWRENCE and (by invitation) MARGARET B. STRINGFELLOW, RICHARD J. ACKART, FRANCIS W. BISHOP and IRVING ARTEL, Rochester, N. Y.

The intravenous injection into normal cats of serum obtained from cats at the height of infectious feline agranulocytosis is followed by leukopenia and pronounced neutropenia in the peripheral blood. Observations of the omental circulation following the intravenous injection of "agranulocytic" serum have been made in an attempt to determine whether the peripheral leukopenia could be explained in the same way as with hydrophilic colloids, i.e. by passage of the white blood cells to the marginal areas with sticking of the cells in large numbers to the endothelial wall. In no instance has there been found increased accumulation of white blood cells in the marginal areas such as has been seen repeatedly following the intravenous injection of 10 per cent gelatin or typhoid vaccine. The results with the serum have varied from practically no change to sudden and prolonged diminution or absence of white blood cells in the para-capillaries of the omentum. In one instance the visible white blood cells disappeared from the omental circulation for a period of 40 minutes. No correlation has been found between the level of the white blood cell count following the injection of "agranulocytic" serum and the number of white blood cells visible in the omental vessels. Since no accumulation of these cells has been noted in the omental vessels, this cannot be accepted as the explanation for the leukopenia. However deposition of cells in other areas has not been excluded. Other observations in our laboratory indicate that peripheral destruction of white blood cells may occur at the height of the disease in infectious feline agranulocytosis. Of course, it is well recognized that the changes in the bone marrow are of major importance with reference to the

leukopenia that occurs at the height of the disease but it is also felt that peripheral destruction may be an additional factor.

Familial Non Hemolytic Jaundice with Indirect Van den Bergh Reaction By WILLIAM DAMESHEK and (by invitation) KARL SINGER, Boston, Mass.

Two families with an unusual type of chronic jaundice were studied. The hereditary nature of the disorder was established in 3 generations. Although the bilirubin was of the "indirect" type suggesting a hemolytic process, the absence of splenomegaly, spherocytosis, reticulocytosis, increased erythrocyte fragility and of a hyperactive bone-marrow was opposed to the diagnosis of familial hemolytic icterus. This condition was furthermore ruled out by the normal or somewhat low values for daily fecal urobilinogen output. The hereditary nature of the disorder and its long duration without symptoms or signs of progressive hepatic disease indicated a simple disturbance in function. A significant abnormality was the greatly delayed excretion of injected bilirubin from the blood, indicating (in the absence of other abnormal tests for liver function) a disturbance in the permeability of the hepatic cells to the excretion of bilirubin. In congenital hemolytic jaundice with approximately the same degree of icterus the bilirubin excretion test was normal. Striking analogies were present between our human cases and the hereditary jaundice of rats recently described by Malloy and Lowenstein. Our studies demonstrated further that familial jaundice is not always hemolytic in type and that an indirect van den Bergh reaction is not necessarily indicative of a hemolytic process.

Treatment of Experimental Canine Black Tongue and Clinical Pellagra with Coramine By DAVID T. SMITH and (by invitation) JULIAN M. RUFFIN, GEORGE MARCOLIS and LESTER H. MARCOLIS, Durham, N. C.

Severe cases of pellagra respond as readily to treatment with coramine (pyridine-B-carboxylic acid diethylamide) as to nicotinic acid. The doses employed by us (3 cc. daily) and by other investigators (2 to 20 cc. daily) were rather large and were chosen empirically without previous animal experimentation.

The experimental method was standardized by treating 45 cases of acute black tongue with daily doses of nicotinic acid which ranged from 0.1 mgm. to 10 mgm. per kilogram of body weight. Daily doses of 0.5 mgm. per kilogram gave the maximum results and no additional improvement resulted from doses 20 times as large.

Coramine was administered parenterally to 22 dogs with acute black tongue in daily doses of 1.45 to 14.5 mgm. per kilogram. Daily doses of 7.25 mgm. per kilogram were uniformly successful and doubling the dose resulted in no additional improvement.

Coramine was administered orally to 20 dogs with acute black tongue. Daily doses of 2.9 mgm. cured 7 out of 8 animals and doses of 7.25 mgm. were uniformly satisfactory.

The minimum dose giving the maximum response may be defined as 7.25 mgm daily per kilogram of body weight.

Patients with acute pellagra should receive 2 to 3 times the dog curative dose or 3 to 5 cc of coramine daily

The Pathogenesis of Azotemia in Hemorrhage from the Upper Gastro-Intestinal Tract By JOHN B JOHNSON (introduced by Samuel H Bassett), Rochester, N Y

Fifteen cases of gastric and duodenal hemorrhage have been investigated in an effort to study the mechanism of this azotemia. Renal function was studied by frequent measurement of the urea and creatinine clearances. In most instances the initial observations of kidney function were begun within a few hours after admission.

No direct correlation between the amount of hemorrhage and azotemia was found. Every case which showed a blood urea nitrogen above 28 mgm. per cent had a definite reduction in renal function. In some cases this reduction was permanent, in others temporary due to shock. None of the cases with normal renal function showed an elevation of blood urea nitrogen above 28 mgm. per cent, even when the hemorrhage was extensive. No changes were observed in the creatinine, CO₂ combining power, and blood chlorides

The conditions of hemorrhage, except for anemia and shock, were simulated in selected cases by the administration of large quantities of human blood by stomach tube. Nitrogen balance studies in these cases revealed that 40 to 60 per cent of the protein given as blood was recovered as nitrogen in the urine. The blood urea nitrogen did not rise significantly above physiological levels except in the cases which showed distinct permanent reduction in kidney function.

Physiological Adjustments of Normal Subjects to Sudden Loss of Blood By EUGENE A STEAD, JR. (introduced by Marshall N Fulton), Boston, Mass

Seven hundred and sixty to twelve hundred and twenty cc. of blood were removed rapidly by venesection from normal professional donors in the horizontal position. Five of the six subjects developed collapse when from 155 to 197 per cent of the total blood volume was removed. As the blood was being removed there was a slight narrowing of the pulse pressure and an increase in heart rate of 14 to 30 beats per minute. The hands and feet became cooler and there was slight sweating of the forehead. During this time the subjects had no cerebral symptoms, the blood flow to the brain being adequate, the carotid sinus and aortic pressor areas caused peripheral vasoconstriction and a rise in heart rate.

The onset of the collapse was sudden. The blood pressure fell sharply to 50 mm. of Hg or below. At the height of the collapse the subjects showed signs of both sympathetic and parasympathetic overactivity. The heart rate was very slow, ranging between 36 and 40 beats per minute. The subjects developed nausea, weakness, ashen gray pallor, blurred vision and profuse sweating. One subject became unconscious.

Immediately after hemorrhage there was a rapid small increase in plasma volume as protein-poor fluid entered the blood stream. This caused a slight fall in the serum protein concentration. The plasma volume continued to increase for 72 hours, but the protein concentration showed no further fall, indicating that protein and fluid were being added to the blood stream at the same time. The fall in hematocrit paralleled the increase in plasma volume.

These observations are important because they show that (1) when dilution is complete on the third to fourth day following venesection, about $\frac{1}{4}$ of the serum protein is protein which has been added to the blood since the hemorrhage, (2) there is no readily available store of non-circulating red blood cells which is added to the blood stream in times of emergency, (3) the peripheral hematocrit reflects fairly accurately the direction and extent of the changes in the plasma volume.

READ BY TITLE

Assay of Adrenocorticotrophic Hormone in Cases of Cushing's Syndrome By KARL E PASCHKIS (introduced by Hobart A Reimann), Philadelphia, Pa.

The syndrome associated with basophilic adenoma of the anterior pituitary, bearing Cushing's name, is clinically nearly identical with certain tumors of the adrenal cortex. It has been assumed that in Cushing's syndrome the pituitary acts by stimulating the adrenal cortex through its adrenocorticotrophic hormone. Serum extracts were assayed for their adrenocorticotrophic activity and were found active in cases of Cushing's syndrome. This finding supports the theory that the basophilic adenoma of the pituitary stimulates the adrenal cortex. In comparison with cases of pituitary basophilism, cases of hypertension and of adrenal cortical tumor were examined. The results were negative.

March Hemoglobinuria: Studies of the Mechanism and Clinical Characteristics By D ROURKE GILLIGAN (by invitation) and HERRMAN L. BLUMGART, Boston, Mass

Hemoglobinuria produced by physical exertion, so-called "march hemoglobinuria," occurs uncommonly. The pathologic physiology of the attacks has not been clearly defined.

Three cases of march hemoglobinuria have been studied by us. The subjects were young males. Brisk short walks in one subject, and running in the other two cases induced attacks. Attacks were asymptomatic. Physical examination revealed no abnormalities except icteric sclerae in two of the subjects, and intermittently palpable spleen and liver in one of these. The urine was completely normal in all cases between attacks. The diagnosis was clearly differentiated from other types of paroxysmal hemoglobinuria.

Hematological findings were completely normal. Hemoglobin was present in the plasma at levels of 40 to 70 mgm. per 100 cc. in each attack of hemoglobinuria. It was calculated that the cells from 7 to 12 cc. of blood

were destroyed during an attack. Less than 10 per cent of the released hemoglobin appeared in the urine. Albuminuria was present at the time of hemoglobinuria. The plasma bilirubin increased considerably after attacks in the two patients with slight jaundice and hyperbilirubinemia.

In the case in which walking produced attacks the body position during the exercise, rather than the degree of exertion, was shown to be the precipitating factor. Thus, when a moderately kyphotic posture was induced by a plaster cast no hemoglobinuria resulted from amounts of walking which regularly produced attacks in the normal upright posture. Further prolonged exercise on the bicycle ergometer with an oxygen consumption greater than that during walking also failed to produce an attack. These findings suggest that interference with blood flow in the abdominal organs during exercise may be important in the production of the hemolysis.

The constant bilirubinemia in two cases was not clearly attributable to blood destruction since attacks of hemoglobinuria were infrequent and there was no anemia or reticulocytosis.

In our experience this condition occurs with sufficient frequency, especially in athletes, so that its presence should be considered in any case in which a red urine is voided.

A Further Investigation of the Uroresin Reaction of Pellagra Urines By J. A. LAYNE (by invitation) and C. J. WATSON Minneapolis, Minn.

The color reaction described by Ellinger and Dojmi was employed by Beckh, Ellinger and Spies as a basis for the quantitative estimation of porphyrin in the urine. These investigators reported marked increases of porphyrin in a series of pellagra urines. Watson observed subsequently that the color reaction was due to uroresin rather than porphyrin. This was confirmed by Meiklejohn and Kark. In the present investigation, additional evidence has been obtained (1) that the color reaction is not due to porphyrin (2) that the chromogen is indolacetic acid, which does not, however give the uroresin reaction except after primary oxidation with nitrite (3) that the chromogen is often present even in the normal urine, but fails to give a spontaneous reaction in the absence of nitrite or of other similar oxidizing substances. The latter have been noted, but not identified, in pathological urines particularly from individuals presenting evidence of nicotinic acid deficiency to a greater or lesser extent.

Although the uroresin reaction is commonly positive in pellagra urines, it has not been possible to correlate its presence and disappearance with the deficiency and the administration, respectively of nicotinic acid. The reaction has often been noted to disappear spontaneously prior to administration of nicotinic acid, and further to reappear even long after adequate amounts have caused regression of all signs of deficiency. Positive spontaneous uroresin reactions (without addition of nitrite) have been noted at one time or another in each of ten

cases of nicotinic acid deficiency studied thus far. It may be emphasized that in none of those was the amount of porphyrin sufficient to be productive of color with the Ellinger Dojmi reaction. This fact could be ascertained readily by preliminary removal of porphyrin from the ether extract of the urine, with 5 per cent HCl. The uroresin color was then developed by extraction from the ether with 25 per cent HCl according to the Ellinger Dojmi procedure. The actual amounts of porphyrin present were not as great as are often encountered in various diseases notably pernicious anemia, lead poisoning and cirrhosis of the liver. There is, therefore, no reason to suppose, as has been suggested, that the light sensitivity in pellagra is related to porphyrin.

The toluene preservatives of pellagra urines, also of urines from certain other patients suffering from malnutrition of one cause or another often develop a pink or even deep red color due in many instances at least to indirubin. The present investigation has sought to determine whether the occurrence of this substance was in any way correlated with the presence of indolacetic acid, indican, or both. No definite correlation has been found to exist. The possibility is not excluded, however that given the proper conditions, indolacetic acid and indoxyl may unite to form indirubin. Certain observations, in fact, suggest that this does occur.

Blood Plasma Volume Changes Following the Administration of Diuretics By GEORGE M. DECHERD JR. and D. B. CALVIN (by invitation) and GEORGE HERRMANN Galveston, Tex.

Patients with congestive heart failure and edema were selected and put at rest in bed under standard conditions for 3 days. The blood plasma volume was determined by the method of Gregerson, Gibson and Stead, as modified for clinical use by Gibson and Evelyn, using the blue dye T1824. After this control determination one of the three usual types of diuretic drugs, salyrgan, aminophyllin or digoxin, was injected and blood samples were taken at frequent intervals during the ensuing 12 hours. Correlation of the plasma volume levels with the urinary output suggests that the mechanism of the diuretic effect is different for each drug.

Salyrgan, intravenously produced plasma volume changes which support our previously held view that the chief site of action of mercurials is on the renal tubular epithelium. There was a decrease in plasma volume which parallels the diuresis. In one instance in which no diuresis followed salyrgan administration there was a moderate increase in plasma volume, suggesting a possible effect on fluid mobilization from the tissue.

When aminophyllin is injected intravenously the present studies indicate that, in addition to the acceleration of glomerular filtration previously described, there is also evident a definite effect elsewhere. This is manifested by a conspicuous rise in plasma volume beginning within 30 minutes after aminophyllin injection. This is abated at the height of the diuretic flow and disappears as

diuresis continues, finally resulting in a sharp drop in blood plasma volume.

After the administration of digoxin there is a slight rise in plasma volume which persists until diuresis is well established, when diuresis occurs the plasma volume drops. The plasma volume seems to be affected by passage of fluid from the tissues, as well as by fluid loss through the kidneys, as would be anticipated when the drug used acts primarily on the myocardium and improves the circulation.

With each of the drugs used, as diuresis progresses there is a mobilization of tissue fluids and electrolytes, with changes in the serum proteins similar to those demonstrated by Calvin in experimental hydremia in the dog.

*Heart Size and Experimental Atheromatosis in the Rabbit** By L. N. KATZ and (by invitation) A. SANDERS, R. S. MEGIBOW, S. CARLEN and J. RANSOHOFF, Chicago, Ill.

The relationship of cardiac hypertrophy to arteriosclerosis and coronary sclerosis is still controversial. This relationship was studied experimentally. Atheromatosis of the aorta and subendothelial lipoidosis of the coronary arteries were induced in 16 rabbits by feeding a high fat and cholesterol diet for three to four months. These rabbits constituted experimental series 'A'. Six rabbits, although on a similar diet for a similar duration of time, developed no gross or microscopic evidence of atheromatosis and these animals constituted control experimental series 'B'. Eighteen untreated rabbits served as controls, constituting control series 'C'. Body weights and ages in all three groups were the same. In series 'C', heart weights varied from 19 to 58 grams, and only two hearts weighed more than 5 grams. The average cardiac weight in this group was 38 grams. Heart weights varied from 21 to 66 grams in series 'B' and the average cardiac weight was 39 grams. Of the 16 rabbits constituting experimental series 'A', there were 12 which had hearts weighing more than 5 grams, and the average heart weight was 63 grams. Pulse wave contours and blood pressure readings were taken in 4 atheromatous rabbits by the direct Hamilton technique, just prior to sacrifice. These were no different from similar determinations made in 7 normal untreated rabbits. Microscopy revealed the presence of partial to complete occlusion of many of the smaller coronary arteries due to marked subendothelial lipoidosis, areas of fatty degeneration, and early and old myocardial infarcts. Apparently, then, marked atheromatosis of the coronary arteries in the rabbit produces cardiac hypertrophy.

The Colorimetric Assay of Weakly Phenolic Ketones (Estrone) in Extracts of Human Urine By N. B. TALBOT, E. MACLACHLAN and F. KARUSH (by invitation) and A. M. BUTLER, Boston, Mass.

Though biological methods for the determination of estrogenic hormones have the advantage of proving the

presence of physiologically active material, they are relatively inaccurate and time consuming. The chemical methods of assaying human urine are, so far as we are aware, applicable only to urine of pregnant women. Any improvement in chemical assay overcoming this limitation and permitting the observation of change in endocrine function would, therefore, be an improvement over existing methods of assay.

The present paper reports a procedure for the determination of the weakly phenolic ketone (estrone) content of human urines which contain as little as 5 or 6 micrograms in the 24-hour output. The urine is hydrolyzed with acid and extracted with ether. The purification procedure of Cohen and Marrion has been modified to include several additional washings with 0.1 N alkali and to include a reducing agent (sodium hydrosulfite) which facilitates the removal of non-estrogenic substances from the extract. The "estrone" fraction thus obtained is further purified by the use of Girard's reagent T which separates ketonic from non-ketonic substances. The ketonic fraction thus obtained should theoretically contain only substances which are weakly phenolic and at the same time ketonic. Thus far estrone is the only example of that type of compound which has been isolated from extracts of human urine. The assay depends upon the formation of a colored compound when estrone is coupled with diazotized diaminidine. This coupling takes place at the phenolic hydroxyl position. No color is obtained, therefore, with non-phenolic ketones such as the neutral 17-ketosteroids (androgens). The extinction wavelength curve of the color developed from solutions of crystalline estrone and pregnancy urine extracts is identical. The data obtained show that

1 Crystalline estrone in amounts ranging from 25 to 30 gamma per sample may be determined by the colorimetric procedure with an accuracy of 10 per cent.

2 The recovery of crystalline estrone in pure solution through the entire purification procedure averages 67 per cent (range 62 to 81 per cent).

3 The recovery of crystalline estrone added to the crude ether extract of hydrolyzed children's urine originally containing no estrone averages 65 per cent (range 57 to 79 per cent).

4 The range of 24-hour excretion of "estrone" for immature children was 0 to 4 gamma.

5 The 24-hour output of "estrone" by normal cyclic women and women in the 4th to 7th month of pregnancy averaged 25 and 400 gamma, respectively.

Thus the data obtained apparently correspond with the physiological status of the individuals studied.

Hypersensitivity to Light Studies on an Unusual Case Treated Successfully with Histamine By RICHARD B. CAPPS and (by invitation) RICHARD H. YOUNG, Chicago, Ill.

Hypersensitivity to light with the production of wheals is a very unusual condition. Such cases as can be found in the literature have been inadequately studied and in-

* Aided by the A. D. Nast Fund for Cardiac Research.

effectively treated. This report concerns an extreme example of this condition with studies of the mechanism and the effect of treatment with histamine. As far as we know similar observations have not been previously made.

The patient, a 34-year-old blond male, was so sensitive to light that as little as $\frac{1}{2}$ of an erythema dose of a carbon arc lamp produced whealing. Fainting occurred with excessive exposure. The effect of light of different wave-lengths was investigated. No porphyrin was present in the urine. An increased histamine content of the blood and an increase in the gastric acidity were demonstrated following whealing. Skin biopsies were obtained before and after exposure.

Accurate studies of the skin sensitivity were made possible by observing the appearance time of wheals with different times of exposure to a standard light. Histamine and the repeated administration of histamine were both found to abolish the reaction of whealing.

Attempts to Produce Pernicious Anemia Experimentally
By MAXWELL M. WINTROBE and (by invitation) HERMAN LISCO JOSEPH L. MILLER, JR. and LAWRENCE R. KOTZ, Baltimore, Md.

Pigs weaned at an early age (3 weeks) were fed a diet consisting of casein (25.8 per cent) sucrose (56.9 per cent) lard (10.8 per cent) cod liver oil (1.3 per cent) and a salt mixture, supplemented with brewers yeast (3 grams per kilogram of body weight).

When they became accustomed to this artificial diet and seemed to be in a good nutritive state, the quantity of yeast given some of the animals was gradually reduced and thiamin, riboflavin, nicotinic acid and filtrate factor were given instead, separately and in various combinations. Comparable animals receiving the above synthetic vitamins and no yeast were given desiccated whole liver or an anti pernicious anemia liver extract. Still others received wheat germ oil or liver in addition to yeast.

Macrocytic anemia occurred in a few animals deprived of yeast but receiving thiamin, riboflavin and nicotinic acid, and in all such animals ataxia and degeneration of the sensory neuron, including the posterior funiculi of the spinal cord, developed. Yeast gave only partial protection against this degeneration but desiccated whole liver or yeast plus wheat germ oil were fully protective.

Assays of the antianemic potency of the livers of ataxic and nonataxic animals have shown distinctly greater potency in the latter.

The Clotting Action of Stock and Detoxified Fer-de-lance Venom By GEORGE L. KAUFER, JR. (by invitation) and PAUL REZNICKOFF New York, N Y

Eagle has shown that snake venoms act as enzymes in promoting the clotting of blood. This study was undertaken to determine the effect of fer-de-lance venom from which the neurotoxin was removed, and to compare its action with a "stock" fer-de-lance venom.

The detoxified venom was first used three months

after its preparation, and the same sample was used throughout the seven-month period of experimentation.

Detoxified venom given intravenously to rabbits in doses of 0.1 cc. of a 1-800 solution to 0.3 cc. of a 1-100 solution caused an increase in the clotting time which lasted three to six hours, varying with the dosage. Preliminary observations indicate that blood fibrinogen is lowest when the clotting time is longest. When given intramuscularly to rabbits in doses of 0.5 to 1.0 cc. of a 1-100 solution, a decrease in the clotting time occurred one to three hours after injection following a temporary increase.

Both stock and detoxified venom clotted oxalated blood much more quickly than heparinized blood. Pure fibrinogen solution was clotted as rapidly by 0.1 cc. of 1-100 detoxified venom as by 0.1 cc. "pure thrombin. The effect of heparin on this reaction is being studied.

Clinical Significance of Urinary Androgens By HARRY B. FRIEDGOOD and JOHN K. WOLFE (introduced by Soma Weiss) Boston, Mass.

Previous studies have disclosed that the range of total daily androgen excretion for normal and virilistic females overlaps, although the latter tend to have higher levels. Excessively high values occur in virilism due to cortico-adrenal tumor and occasionally in virilism without tumor. Estimation of the total urinary androgens is not clarifying the nature of the pathological physiology of virilism nor is it useful, except in a limited way in the differential diagnosis of these cases. These considerations led to a study of the chemical structure of the urinary androgens and the relative proportions in which they are excreted in two cases of virilism due to tumor and one case of adrenogenital syndrome without tumor.

Two crystalline compounds have been isolated and positively identified chemically viz. dehydroisoandrosterone (I) and Δ 3,5-androstadiene-17-one (II) (I) which has been isolated in all cases, accounts for 45 to 50 per cent of androgenic activity in one tumor case, 13 per cent in another and 1 to 2 per cent in the adrenogenital syndrome. (II) which was found in the first tumor case, and is being sought in the others, represents the first isolation of this androgen from female urine. The remaining keto-steroids, still unidentified, are being subjected to a similar systematic study.

The Peripheral Blood Flow in Hyperthyroidism. By HAROLD J. STEWART and (by invitation) WILLIS F. EVANS, New York, N Y

Measurements of the peripheral blood flow in cc. per sq. m. per minute have been made in patients suffering from Graves disease. In addition, the basal metabolic rate, velocity of blood flow (arm-to-tongue method (de-cholin)) pulse rate, and blood pressure were recorded in each of three phases studied namely before iodine, again during iodine therapy and finally after thyroidectomy. Eighteen patients have been studied. In the measurement of the peripheral blood flow we made use of the Hardy-Soderstrom radiometer and the methods described

by Hardy, Daniel, and Soderstrom All measurements were made in the morning while the patients were in a basal metabolic state, the room temperature did not vary more than 0.5° C in any single experiment, the subjects were nude, being covered only by a sheet Seventeen patients were women and one a man The ages ranged from 18 years to 56 years In the eight patients upon whom these studies have been completed, it was found that the peripheral blood flow was high (average 213 cc. per M² per minute) in the phase "before iodine" when the basal metabolic rate was elevated After the institution of iodine therapy, decrease in peripheral blood flow (average 139 cc. per M² per minute) and in basal metabolic rate occurred After thyroidectomy, a further decrease in blood flow (average 78 cc. per M² per minute) and in basal metabolic rate was recorded The relationship between basal metabolic rate and peripheral blood flow was linear in that, when all the observations were pooled, it was found that as the basal metabolic rate decreased, the peripheral blood flow decreased Definite but not striking increase in circulation time occurred, approaching the normal after thyroidectomy Pulse rate and blood pressure usually followed the basal metabolic rate and peripheral blood flow The skin in most cases showed an increased average skin temperature before therapy and decreased with iodine and thyroidectomy No significant fluctuations in rectal temperature were observed There are certain observations indicating that the minute volume output of the heart is increased in Graves' disease, and now from these observations it appears that the peripheral blood flow is also increased

Renal Enlargement in Rats Produced by Testosterone Propionate By JOHN B LUDDEN and ERICH KRUEGER (by invitation) and IRVING S WRIGHT, New York, N Y

Selye reported renal enlargement in *adult female mice* and rats after testosterone propionate The present study of the effect of testosterone propionate on *immature male, mature male and female* rats demonstrates similar action in each group The histological picture varied from that of Selye.

Studies were made on *immature male* rats (12.5 mgm. testosterone propionate daily for 14 days), *mature male* rats (10 mgm. testosterone propionate daily for 21 days) and *mature female* rats (10 mgm. testosterone propionate daily for 21 days) Controls were given sesame oil

Average weight of kidneys of treated *immature male* group was 831 mgm. per 100 grams of body weight compared with 691 mgm. in controls Kidneys of treated *mature male* group had average weight of 993 mgm. per 100 grams of body weight compared with 697 mgm. for controls In *mature female* group average kidney weight was 933 mgm. per 100 grams of body weight compared with 755 mgm. in controls In mature groups differences were especially clearcut, there being no overlapping of kidney weights of treated rats by those of control rats.

Histological study of kidneys revealed generalized enlargement in all groups, but no other clear evidence of

deviation from normal morphology This observation contrasts with findings reported by Selye, namely, definite hypertrophy of parietal lamina of Bowman's capsule.

The Role of Anemia in Water Retention By MAURICE B STRAUSS and (by invitation) HERBERT J FOX, Boston, Mass

Since Addison first noted the occurrence of edema in the anemia now bearing his name, it has been recognized that water retention is of common occurrence in various types of anemia This phenomenon has been ascribed to "cardiac weakness" However, venous pressure determinations have failed to reveal abnormal levels Although some patients with anemia may have lowered plasma protein levels, no correlation of the two factors was observed by Keefer and Myers nor by us in 32 pairs of observations When sodium salts were administered to these 32 patients, water retention occurred in all, the degree of retention varying directly with the severity of the anemia No significant correlation between the amount of water retention and the colloid osmotic pressure of the plasma proteins was observed It is concluded that anemia *per se* is conducive to water retention and edema formation

Electrocardiographic Changes During Intravenous Therapy of Pneumonia (Preliminary Report) By DAVID D RUTSTEIN, K JEFFERSON THOMSON, DANIEL M TOLMACH and ROBERT J FLOODY (introduced by L Whittington Gorham), Albany, N Y

As part of a study of the circulation of pneumonia patients, electrocardiographic observations in relation to intravenous therapy were made.

Electrocardiograms of pneumonia patients were taken prior to intravenous therapy During intravenous administrations, chest lead IV F (electrode position constant) was observed in a "cardioscope." Tracings were recorded at about the middle of each injection, two minutes following its completion, and whenever changes appeared during administration These consisted of variations in direction, configuration, and voltage of T₁ and P₁.

In 59 pneumonia patients, 112 injections of horse or rabbit serum (49 horse and 63 rabbit) were studied The electrocardiographic variations following rabbit serum were similar to those following horse serum The electrocardiograms in 19 (32.2 per cent) of these patients changed during or after at least one injection Twenty-three (20.5 per cent) of the 112 administrations were accompanied by a variation 16 in T₁ and 7 in P₁.

A similar study was conducted on 24 pneumonia patients who received 32 injections of sodium sulfapyridine or sodium sulfathiazole (26 sodium sulfapyridine and 6 sodium sulfathiazole) Five (20.8 per cent) of these injections in 5 (15.6 per cent) of the patients, were associated with a change 3 in T₁ and 2 in P₁ Six patients had nausea or vomiting during the intravenous administration of one of these drugs Only 2 of these had electrocardiographic changes during the occurrence of

these symptoms one had inversion of P_1 and the other a disappearance of P_1 associated with a slowing of the rate.

Electrocardiographic changes indistinguishable from acute myocardial infarction were observed in 3 patients. One received Type I antipneumococcus horse serum, an other Type VIII antipneumococcus rabbit serum, and the third sodium sulfathiazole.

Varying Relations Between Inulin Creatinine and Urea Clearances in Children with the Nephrotic Syndrome
By LEE E. FARR and (by invitation) PALMER H. FUTCHER and KENDALL EMERSON JR. New York N Y

In approximately one-fifth of the children under five years of age admitted to this clinic with the nephrotic syndrome, the urea clearance was increased to above 140 per cent of normal. With recovery from the disease, the urea clearance dropped to normal levels. We have seen this type but once in an adult. In the remaining four fifths of the nephrotic children under five years of age and in the great majority of our nephrotic patients over this age, the urea clearance was normal or sub-normal on the patient's first admission. The present studies were carried out to determine if in these two groups of patients, the renal clearance of two non urea substances was affected in the same manner as urea.

Inulin, exogenous and endogenous creatinine, and urea clearances were determined simultaneously in several high clearance nephrotic children with urea clearances 140 to 200 per cent of normal, and in several low clearance nephrotic children with urea clearances below 20 per cent of normal. The duration of disease was similar in comparative patients in each of the groups.

In patients with high urea clearances the inulin clearance was found to be increased above the normal ranges to about the same degree as the urea clearance. On the other hand, the endogenous creatinine clearance was substantially below the inulin clearance.

In patients with low urea clearances, all three clearances tended to approximate closely the same absolute value.

The significance of the above observations is discussed.

Studies on Migraine The Contrast of Vascular Mechanisms in Headache and Pre-Headache Phenomena. By G. A. SCHUMACHER and A. M. CAHAN (by invitation) and H. G. WOLFF New York, N Y

Headache

It has been shown that migraine headache results from the dilatation and stretch of cranial arteries. To ascertain further the role of the cerebral arteries in migraine headache the following was done. During severe headache in 5 subjects lumbar puncture was performed. By means of a manometric system attached to the lumbar needle, the cerebrospinal fluid pressure was progressively increased to approximately 800 millimeters of water. Such increase in pressure, sufficient to abolish histamine

headache, did not diminish the intensity of the headache. It is inferred that the headache in migraine does not arise primarily from the cerebral arteries, but chiefly from the dilatation and stretch of the branches of the external carotid artery.

Pre-headache phenomena—scotomata

Perimetric studies of pre-headache scotomata were correlated with systemic arterial pressures during the action of amyl nitrite. (1) After small amounts of this agent, the scotomata diminished and disappeared within 10 seconds after the facial flush to remain absent for 2 to 4 minutes. During visual restoration there was little change in the systemic arterial pressure. (2) After the inhalation of larger amounts of amyl nitrite, the scotomata again promptly disappeared shortly after the flush, to be soon followed, however by confluent scotomata which merged to produce, except for central vision, transient amaurosis. This was associated with disorientation and pronounced fall in blood pressure. Normal visual fields again followed and then the scotomata reappeared. It is inferred that these pre-headache phenomena result from cerebral vasoconstriction which is succeeded by the aforementioned vasodilatation and headache.

Determination of the Transfusion Requirement in Anemia By JOHN G. GIBSON 2ND (introduced by Henry A. Christian) Boston, Mass.

Every clinician has wanted to know how much blood must be given to an anemic patient to raise the hematocrit to the desired level. In the past, this information could be obtained only by actual determination of the blood volume. The transfusion requirement can now be quickly computed from the patient's hematocrit and predicted normal total red blood cell volume (obtained from a nomogram).

In chronic anemia there is a direct relationship between the percentage of reduction from normal in total red cell volume (deficit) and the hematocrit. Thus, at hematocrit levels of 10 20 30 and 35 total red cell volume is reduced by approximately 75 50 30 and 15 per cent, respectively. The product of the difference in percentage deficits corresponding to the original and desired hematocrit level (obtained from a nomogram) and the normal total red cell volume, divided by the average hematocrit of transfusion blood (about 40) gives the amount of whole blood needed to attain the desired hematocrit level. Excess plasma given by transfusion is quickly disposed of.

Normal total red cell volume varies greatly with sex and physical measurements, ranging from 1200 cc. in small females to 2860 cc. in large males. In anemic patients with normal total red cell volumes of these amounts, the minimum quantity of whole blood required to raise the hematocrit from 20 to 30 is from 500 cc. to 1500 cc.

The computation is not valid in acute hemorrhage before dilution has taken place (2 to 4 days) or in shock, in which there may be hemococoncentration.

Application of Clearance Method to Determination of Unilateral Renal Blood Flow in Man By HERBERT CHASIS, JULES REDISH and ALBERT ERDMANN, JR. (introduced by William S. Tillett), New York, N. Y.

In view of numerous reports indicating that unilateral renal disease may be a frequent etiological factor in essential hypertension, a more accurate method of appraising unilateral renal function is required. To this end the clearance method has been applied to the unilateral measurement of renal blood flow, filtration rate, etc., in a limited group of patients with normal renal function, with demonstrated unilateral renal disease, and with essential hypertension. It has been demonstrated that accurate simultaneous collection of right and left ureteral urine is possible if necessary precautions are taken to observe and prevent leakage around the catheters. The total renal blood flow determined in this manner is comparable to the figure obtained by the usual total clearance method. In those patients with essential hypertension who have been examined thus far, the renal blood flow was found to be equal in both kidneys, though the filtration fraction is characteristically increased above normal. In the patients with unilateral uropathy the blood flow was found to be decreased in the diseased kidney. In two patients who had operative procedures designed to increase renal blood flow (renal omentopexy in one and nephropexy for ureteral kink in the other), renal blood flow was found to be lower in the operated kidney two years later.

The method is now being applied to the measurement of the tubular excretory mass (D-Tm), i.e., the evaluation of the total functional renal mass.

Effect of the Application of Tourniquets on the Hemodynamics of the Circulation By RICHARD V. EBERT (introduced by James P. O'Hare), Boston, Mass.

Many clinicians believe that pooling of blood in the extremities by means of tourniquets is as effective as phlebotomy in the treatment of acute left ventricular failure. The purpose of this study was to determine whether the amount of blood pooled in the extremities by this means was equal to that removed by the usual phlebotomy. The blood volume was determined in 5 subjects under the following conditions: (1) with the extremities free, (2) after occlusion of the arterial circulation to both legs and one arm by cuffs inflated to a pressure of 250 mm. of Hg, (3) after venous congestion of the three extremities by inflating the cuffs to diastolic pressure for 7 to 10 minutes, followed by occlusion of the arterial circulation. By subtracting the result obtained in Experiment 2 from that obtained in Experiment 1, the volume of blood normally contained in the three extremities was calculated. By subtracting the result obtained in Experiment 3 from that obtained in Experiment 2, the volume of blood which was removed from the head, trunk and arm by venous congestion of the remaining three extremities was calculated.

In 5 normal subjects in the recumbent position the average volume of blood contained in the three extremi-

ties was 900 cc., or 16 per cent of the total blood volume. An average of 740 cc. of blood was removed from the head and trunk by congesting the extremities. Therefore, tourniquets effectively applied pool more blood in the extremities than is removed from the body by the average venesection and are a rational therapeutic measure.

The sudden removal of an average of 15 per cent of the volume of blood circulating in the head and trunk (740 cc.) by tourniquets produced circulatory collapse in 4 of 7 normal subjects tested. This agrees with the observation that the rapid removal of 15 to 20 per cent of the blood volume in man by venesection is accompanied by the symptoms of shock.

Proteus and Pyocyanus Infections: a Review of Six Cases of Bacteremia with Immunologic Studies in One By GUSTAVE J. DAMMIN (introduced by Clifford L. Derick), Boston, Mass.

The mortality rate in *Proteus* and *Pyocyanus* bacteremias which result from dissemination from a primary focus is high. When this focus is the kidney, the mortality rate is about 60 per cent. In 5 of the 6 cases in this series, the kidney was the primary focus. Recovery occurred in but one of these cases.

From the recovered case, *B. pyocyanus* (*Pseudomonas aeruginosa*) and *Proteus mirabilis* were isolated from the blood, urine, stool and osteomyelitic sinus. The patient entered in peripheral vascular collapse with bronchopneumonia and right kidney blocked by calculus. Nephrostomy and subsequent nephrectomy were performed. Otherwise treatment was symptomatic. By agglutination, mouse protection and bactericidal tests, a high degree of immunity was demonstrable. The agglutinin titers for these organisms from all the sources varied for *B. pyocyanus* between 1:80 and 1:10,000 and for *Proteus mirabilis* between 1:2560 and 1:80,000. White mice were protected against more than 2 MLD of both organisms. Bactericidal antibodies for *B. pyocyanus* were present in higher titer than for *Proteus mirabilis*, showing a bactericidal action 1000 times greater than the control.

A positive Weil-Felix reaction appeared during the three-months' course of investigation. By agglutinin absorption tests, this strain of *B. Pyocyanus* and *Proteus* X19 were demonstrated to contain a common antigen.

The Effects of Ingestion of Large Amounts of Fluid Upon the Course of Circulating Blood Volume By EMANUEL GINSBURG (by invitation) and SAMUEL H. PROGER, Boston, Mass.

Reports concerning the immediate effects of ingestion of fluids upon the circulating blood volume are conflicting. Accordingly, the problem was reinvestigated, the course of the blood volume being followed for 3 hours after drinking 1000 cc. of water or 1 per cent saline. The improved method for determining plasma volume by means of the "Evans Blue" dye was used. Blood pressures were also followed. Nine individuals without car-

diovascular disease, most of them schizophrenic, served as subjects. There were four experiments with water and seven with 1 per cent saline. Of ten patients with cardiovascular disease, eight received salt solution and two received water.

In the group without cardiovascular lesions there was a 9 per cent drop of both circulating plasma and total blood volumes 15 minutes following the ingestion of 1000 cc. of saline. In three of four patients in the same group there were no changes in 15 minutes after the ingestion of 1000 cc. of water. In this entire group the circulating blood after 1½ hours had not increased above the preingestive values.

The course of the blood volume in patients with cardiovascular disease was parallel to that in the preceding group except that there was no difference between the effects of salt and water 15 minutes after ingestion.

The arterial blood pressures 15 minutes following ingestion were elevated in 50 per cent of all cases comprising the two groups, and unchanged in the others. During the remainder of the experiments, there were no changes from the preingestive values.

Patients' Attitudes and Behavior in Ward Round Teaching By JOHN ROMANO (introduced by William P. Murphy) Boston, Mass.

Bedside teaching is an established procedure in medical education. However patients' reactions to this procedure have received little attention. In order to gain objective data, an investigation was undertaken (1) to discover if the experience is traumatic, (2) to understand the patients' reactions to their illnesses, (3) to study the patients during presentation, which is an experimentally induced anxiety situation, (4) to learn how the procedure may be utilized as psychotherapy.

Accordingly under uniform conditions, 84 unselected patients were studied before, during and after Saturday morning rounds. Three to 5 patients were presented to the resident and visiting medical staff and to 40 to 60 students and visiting physicians. Methods of examination before, during and after presentation included psychiatric study, determination of pulse, respiration, blood pressure, spontaneous discussion by the patient of his reactions. The patients were classified as having (1) little or no anxiety associated with their physical disease, (2) considerable anxiety with their physical disease, (3) predominantly neurotic symptoms with no physical disease, (4) confusion due to various factors.

No severe psychological trauma was observed. In the few instances in which patients were tense or anxious, the reactions were mild and in no way resembled panic reactions.

Considerable information was obtained concerning the emotional significance of the illness to the patients. Anxiety was experienced by less than one-fourth of the patients in the period preceding presentation. 14 patients exhibited objective evidence of tension during presentation. 12 were embarrassed by the public recitation of the history. 6, by the examination. There were

no essential changes in pulse, respiration, blood pressure.

While the entire procedure of the ward rounds may and should be utilized as psychotherapy the discussion period has the greatest potential value. Most patients preferred to remain for discussion. Many patients requested that the essential conclusions of the conference, particularly as they related to their illness, recovery and return to work, be communicated to them in simple understandable language.

Intravenous Injection of Magnesium Sulfate in Subjects With Hypertensive and Renal Disease By ALEXANDER W. WINKLER and (by invitation) HERBELL E. HOFF and PAUL K. SMITH New Haven, Conn.

The response of patients with cardiovascular and renal disease to intravenous injection of magnesium sulfate differs in certain respects from that of normal subjects. Normally injection of small amounts of magnesium sulfate evokes intense cutaneous vasodilatation accompanied by marked fall of blood pressure. In hypertensive subjects cutaneous vasodilatation was regularly observed, but simultaneous fall in blood pressure was frequently slight or altogether absent.

Injected magnesium is normally excreted rapidly and almost completely in the urine, while in subjects with impaired renal function urinary excretion is much delayed. With advanced renal insufficiency the concentration of magnesium in the serum may remain elevated for several days, since little is excreted by the gut.

Therapeutic injection of 500 cc. of 2 per cent solution raises the serum concentration to about 7 mEq per liter which is insufficient to produce demonstrable change in the nervous system except relief of headache. Another injection within forty-eight hours raises the concentration to about 12 mEq per liter because of the persistent elevation caused by the first injection. At this higher level drowsiness and depression of tendon reflexes appear. Respiratory depression occurs only after disappearance of tendon reflexes. Thus these reflexes are a guide during injection.

A New Hypothesis of the Production of the T-wave in the Electrocardiogram Based on Electrokinetic Phenomena. By J. ROSCOE MILLER and ROY F. DENT (introduced by N. C. Gilbert) Chicago III.

Perfusion experiments on the dead heart showed that a potential was developed between the epicardium and endocardium. With a pressure of 40 cm. of mercury an amount of current in excess of one half millivolt was produced. This indicated that the intramural streaming potentials incident to cardiac contraction were sufficient to account for the T wave in the electrocardiogram. Consequently an attempt was made to reproduce the T wave by forcible constriction of the dead heart. Experimental animals were killed by means of intravenous injection of magnesium sulfate. This lowered the irritability of the myocardium so that a short time after the chest was opened there was no response to stimulation. Manual constriction of the heart produced a T-wave

similar to that seen in the unanesthetized animal during normal cardiac contraction. The contour of the wave could be regulated by the amount of pressure exerted and the length of time it was continued. These facts are significant in that they indicate that the T-wave is a product of cardiac contraction and contradict the current explanation based on the theory of repolarization.

A Study of the T-Wave of the Electrocardiogram in Left Bundle Branch Block By WILLIAM A. SODEMAN, New Orleans, La.

T₁ is typically inverted in left bundle branch block, but at times is "atypical" (isoelectric, diphasic, or upright). To determine the significance of such changes, tracings were selected showing normal conduction and bundle branch block in the same patient. With the aid of a planimeter, determinations of the areas of the QRS complex and T-wave in microvolt seconds were made for both types of conduction. Comparison of the summation of the QRS and T values with both types of conduction confirmed the observations of Wilson and further indicated that the "atypical" T-waves in bundle branch block could be predicted and were the expected findings upon the basis of changes in the QRS area. With block negative T-waves became more negative, less negative, and even positive, upon the basis of changes in the QRS alone. Previously positive T-waves gained in negativity depending upon the changes in QRS area. The results indicate that (1) T-wave changes resultant from left bundle branch block bear a definite relation to the QRS and T areas in normal conduction, (2) "atypical" T-wave changes (upright, diphasic) in Lead I do not necessarily indicate further myocardial changes, and (3) the "typical" (inverted) T-wave may be secondary to QRS changes, may result from local variations in the excitatory process resulting from disease, or both.

Culture of Human Marrow Studies of the Relative Effectiveness of Neorsphenamine, Mapharsen, Sulfanilamide, Sulfapyridine, Sulfathiazol, and Sulfamethylthiazol on Infections with Streptococcus Viridans (Alpha Hemolytic Streptococcus) By EDWIN E. OSGOOD and (by invitation) INEZ E. BROWNLEE and JULIA JOSKI, Portland, Ore.

Marrow cultures were infected with streptococcus viridans, and equal portions were placed in each of several vials to each of which, except the control, was added the desired concentration of the drug to be tested. Pour plate colony counts and stained smears were made at intervals. Neorsphenamine in a concentration of 1-150,000 was effective against more strains than any other drug tested, but it had to be present for six to forty-eight hours in this concentration. Sulfathiazol in a concentration of 1-10,000 was effective against many strains including those against which neorsphenamine was ineffective, but was ineffective against some strains against which neorsphenamine was effective. Sulfamethylthiazol and sulfapyridine were effective against most of the strains which were sterilized by sulfathiazol,

but because of their lower solubility would probably not be as effective clinically. Sulfanilamide was ineffective against the strains tested, and mapharsen in a concentration of 1-1,500,000 was effective against some strains but not as effective as neorsphenamine in a concentration of 1-150,000.

A suggested plan of treatment for subacute bacterial endocarditis based on these studies and the clinical results to date are presented with lantern slides which illustrate these results.

Results of Treatment of Diabetes Insipidus in Man and in Animals with Pellets of Desiccated Posterior Pituitary Gland and with Pitressin in Oil By JAMES A. GREENE and (by invitation) L. E. JANUARY, Iowa City, Ia.

Methods to produce slow absorption of hormones are desirable in certain endocrine deficiencies. Diabetes insipidus is such a disease. Five cats with experimentally produced diabetes insipidus and two patients have been treated by implantation of pellets of desiccated posterior pituitary gland. Three such cats and three patients have been treated with injections of pitressin in oil.

The diabetes insipidus has been controlled by such therapy in the animals and in the patients. Certain difficulties encountered with pellet therapy are discussed. The use of pitressin in oil indicates that it is of definite value in the control of this disease.

The Effect of Undernutrition on the Ovary of the Guinea Pig By D. J. STEPHENS and (by invitation) WILLARD ALLEN, Rochester, N. Y.

In a previous communication it has been shown that undernutrition in the guinea pig results in thyroid atrophy with flattening of the acinar epithelium and accumulation of colloid. The thyroids of such animals were unusually responsive to stimulation by the thyrotropic factor of the anterior hypophysis. The ovaries of the undernourished animals also showed evidences of atrophy and retrogression. Underfeeding sufficient to result in a loss in weight of 25 to 30 per cent in a period of two weeks resulted in reduction of ovarian weight and in marked retrogressive changes. There was virtual disappearance of follicles in the majority of the animals. The interstitial cells of the ovaries of the undernourished animals were small and their nuclei showed the "cartwheel" formation of chromatin which has been described in hypophyseal insufficiency. Refeeding resulted in increase in body and ovarian weight and a return of ovarian histology toward normal. The administration of small amounts of an anterior pituitary extract resulted in marked hypertrophy and hyperplasia of the interstitial tissue and increase in ovarian weight.

Evidence is presented which suggests that the retrogressive changes occurring in thyroid and ovary in undernutrition may be due to failure of the anterior pituitary to continue to produce thyrotropic and gonadotropic substances in amounts sufficient to maintain thyroid and ovarian structure and function.

Studies on the Nicotinic Acid Content of Blood and Urine By E. WHITE PATTON and W. R. SUTTON (by invitation) and JOHN B. YOUNG, Nashville, Tenn.

A study has been made of the nicotinic acid content of blood and urine, utilizing the cyanogen bromide-aniline reaction as a basis for the chemical determinations. Values obtained without initial hydrolysis have been compared with those obtained after mild and strong hydrolysis.

In normal subjects the range of nicotinic acid concentration has been found to be 0.30 to 0.50 mgm. per cent in the blood and 30 to 50 mgm. are excreted in twenty-four hours in the urine. Subjects with known nicotinic acid deficiency have exhibited a slightly lower blood level and a definitely lowered urinary excretion of nicotinic acid. The variation in the blood level in these subjects has not been consistent.

Varying degrees of alkaline hydrolysis of the blood yield results which are very similar to those obtained without initial hydrolysis. In urines with a normal or low nicotinic acid content, varying degrees of hydrolysis resulted in a distinct increase over the value obtained without initial hydrolysis. This increase was not noted when urines with a high concentration of nicotinic acid were similarly treated.

Oral administration of 150 to 300 mgm. of nicotinic acid or nicotinic acid amide causes a variable and transitory rise in the blood level within one hour. When daily doses of this magnitude were taken orally the blood level rose to approximately twice its previous level. In deficient subjects there seems to be some lag in this blood level elevation after repeated daily doses of nicotinic acid. Within three hours approximately 20 per cent of the ingested dose of nicotinic acid may be accounted for in the urine of normal subjects, whereas only about 3 per cent of a similar dose of nicotinic acid amide may be recovered in a similar time. In each instance the increase in urinary excretion subsequent to oral administration is transitory and terminates within three hours after ingestion.

Erythrocyte Resistance in Congenital Hemolytic Jaundice and in Experimentally Produced Jaundice By RICHARD T. BEEBE and (by invitation) ABRAHAM FALK, ALBERT M. YUNICH and EDWARD P. HANLEY Albany N. Y.

In the course of some investigations on the fragility of erythrocytes in disease we were impressed by the striking increase in resistance of the red blood cells in patients with obstructive jaundice. We were able to bring about this condition in dogs by ligating the bile ducts and showed that there was a direct relationship between the degree of jaundice and the fragility of the cells. We were unable to demonstrate increased resistance of erythrocytes when jaundice was produced in rabbits by injecting bile salts and bile pigment.

Red blood cell resistance is also increased in chronic secondary and primary anemias without jaundice, leading

us to feel that bile pigment is not the important factor in causing the increased resistance of red cells.

We hoped, on the basis of this and other observations to be able to bring about an increase in the resistance of cells in hemolytic jaundice by the administration of some factor in bile, but as yet have been unsuccessful.

A Comparison of Certain Vascular Responses of Normal and Hypertensive Rabbits By K. K. FRIEDLAND and F. KAPP (by invitation) and E. M. LANDIS Philadelphia, Pa. and Charlottesville, Va.

It has been shown previously in normal rabbits (Landis, Montgomery and Sparkman, J. Clin. Invest. 1938 17 189) that when tyramine, epinephrine, guanidine and pitressin are injected in doses sufficient to elevate blood pressure they also diminish blood flow to the ear as measured by fall of skin temperature. In contrast, heated extracts of rabbits kidneys elevate blood pressure conspicuously without diminishing auricular blood flow.

In the present observations the vascular responses of hypertensive rabbits were studied by the technique used previously for studies on normal rabbits. Hypertension was produced by applying to both renal arteries silver clips channelled to a depth of 0.5 to 0.6 mm. The auricular vessels of these hypertensive rabbits dilated when body temperature was elevated to between 40 and 40.5 C., as was the case with normal rabbits. The amplitude of arterial pulsation in the ear was greater than in normal rabbits but skin temperature rose to the same point in both groups during maximal dilatation. Epinephrine and tyramine raised blood pressure and diminished peripheral blood flow in hypertensive and normal animals but with conspicuous differences in sensitivity. Rabbit kidney extracts raised blood pressure of the hypertensive rabbits in spite of the higher resting pressure prior to injection, but the skin temperature of the ear did not change. It appears that the temporary pressor response to kidney extract is merely superimposed upon the existing hypertension.

Penetration of Clot by Sulfanilamide Sulfapyridine Sulfathiazole and Sulfamethylthiazole By JAMES M. FAULKNER and (by invitation) CHARLES N. DUNCAN Boston, Mass.

Solutions of sulfanilamide, sulfapyridine, sulfathiazole and sulfamethylthiazole were prepared in normal saline and in human blood serum in concentrations varying from 10 to 24 mgm. per 100 cc. In these solutions human blood clots were suspended for periods of 24 to 240 hours, at 37.5 C. At the end of the period the clot was removed and washed with distilled water and chemical determinations were made of the amount of drug present in the clot and in the surrounding solution. It was found that there was no appreciable penetration of the clot by any of the compounds. On the other hand, when whole blood containing any of the above compounds was allowed to clot, the drug was distributed approximately evenly between the clot and the serum. If

is to be drawn from these observations relative to the chemotherapy of subacute bacterial endocarditis, it is that, even if the drug is effective when in contact with the causative organism, rapid eradication of the infection is not to be expected. However, it seems possible that, if treatment is continued over a long enough period of time, preexisting thrombus may become organized and all new thrombus laid down will be impregnated with the drug, thus gradually bringing about a less and less favorable medium for growth of the infecting organism.

The Action of Dihydrotachysterol (A T 10) on the Concentration of Serum Sodium, Protein and Calcium

By JOHN H. TALBOTT and (by invitation) WALTER F. LEVER, Boston, Mass

Metabolic studies on more than 50 patients suffering from pemphigus have been performed during the past 5 years. In most patients a decreased concentration of serum sodium, protein and calcium accompanied the cutaneous lesions. These findings lend weight to the argument that the pathogenesis of symptoms in pemphigus is associated with a disturbance of the acid-base equilibrium in the body.

In searching for some measures to restore the disordered equilibrium, A T 10 was considered because of its serum calcium raising effect. This was achieved according to expectations in each of 12 patients to whom it was given. Other effects not anticipated included an increase in concentration of serum sodium and an increase in concentration of serum protein. With the doses recommended, the average increase in serum calcium was 2.3 m.eq. per liter (46 mgm. per 100 cc.), the average increase in serum sodium was 5.6 m.eq. per liter, and the average increase in serum protein was 2.5 grams per 100 cc. Clinical improvement followed chemical restoration.

It is hoped that these properties of A. T. 10 may be utilized in the treatment of other conditions such as elevation of serum protein in patients with hypoproteinemia.

Comparison of the Action of Choline and Lipocain in the Prevention of Cholesterol Atherosclerosis in the Rabbit By K. R. ANDREWS (by invitation) and G. O. BROWN, St. Louis, Mo

In 1937, Huber, Casey and Brown showed that the pancreatic extract lipocain prepared according to the method of Dragstedt, Van Prohaska and Harms was effective in preventing atherosclerosis in rabbits fed a diet rich in cholesterol.

Steiner in 1938 showed that choline in a daily dosage of 500 mgm. also had the effect of preventing cholesterol atherosclerosis in the rabbit.

All lipocain which we have so far prepared contains at least some choline. The present study compares the preventive action in cholesterol atherosclerosis of choline in dosages of 500 mgm., 75 mgm., 40 mgm., and 10 mgm. per day with daily dosage of lipocain of similar choline content.

The results indicate that choline exerts some protective action in dosages much lower than those used by Steiner. We have found some variation in potency of different preparations of lipocain. One preparation fed in rather large bulk to secure a high choline intake gave very poor protection. Other preparations of much lower choline content gave good protection.

The results to date have indicated that the potency of lipocain preparations may in large part be attributed to the choline content, but do not exclude the possibility that some other substance may also be operative.

Further Studies on the in Vitro and in Vivo Dissolution of Calcium Phosphate Urinary Calculi By FULLER ALBRIGHT and (by invitation) HIRSH SULKOWITZ, Boston, Mass

In 1939 we reported that sodium citrate citric acid solutions have a marked ability to dissolve calcium phosphate calculi. The present study is concerned with an analysis of the properties of solutions which influence their effectiveness for this purpose.

It was found that rabbits' teeth were sufficiently uniform in structure so that their loss in specific gravity on exposure to a solution could be used as a "measuring stick."

The following observations were made: (1) Weakly dissociated acids buffering between pH 4.0 to 4.8 (*i.e.* pK' 5.0 to 5.8) are the most effective. (2) At a given pH, the rate of solubility parallels the amount of undissociated acid (*i.e.* the $\frac{\text{acid}}{\text{salt}}$ ratio). (3) In equimolar solutions, dicarboxylic acids with pK' approaching pK', are more efficient than monocarboxylic acids. (4) When the $\frac{\text{acid}}{\text{salt}}$ ratio is kept constant, the rate of solubility in the pH range 3.0 to 4.8 is approximately constant and relates itself to the phosphoric acid titration curve. (5) The following organic acids were studied and are listed in the order of their efficiency: citric, phthalic, succinic, laevulinic, beta-hydroxybutyric, acetic, malonic, propionic, gluconic, lactic and pyruvic.

The usefulness of an acid clinically will depend on its ability to dissolve calcium phosphate stones without causing irritation. Several acids have been found which are almost as efficient as citric and less irritating.

Studies on the Mechanism of Hemolysis in Preserved Human Blood By ELMER L. DEGOWIN and (by invitation) JOHN E. HARRIS and JOY BELL, Iowa City, Ia.

Studies on the manner in which dextrose inhibits hemolysis are useful in devising new methods of preserving blood. Experimental data are presented to support the following conclusions: Isosmotic concentrations of dextrose and sodium citrate solutions are not isotonic for erythrocytes immediately after collection of the blood. Furthermore, there is a slowly progressive swelling of the red corpuscles during storage in the refrigerator over a period of 30 days. The amount of cell swelling during storage is not directly correlated with the amount of

hemolysis. Dextrose solutions do not inhibit hemolysis by supplementing the sugar lost by glycolysis. Within certain limits, dextrose inhibits hemolysis by serving as a non-electrolyte diluent of plasma rather than by any specific action of the sugar. Studies with sucrose solutions confirm this. The swelling of the cells in dextrose account for the increased fragility in hypotonic saline solutions this swelling is, to some extent, reversible.

During storage, the plasma sodium diffuses into the cells as the potassium goes out. The addition of substances known to be enzyme poisons throws some light on the nature of the hemolytic process.

Iodine Components of the Blood Circulating Thyroglobulin in Normal Persons and in Persons with Thyroid Disease. By J. LERMAN Boston, Mass.

Rabbits injected with human thyroglobulin by various routes particularly according to the method of Dienes (*J. Immunol.*, 1923, 15:141) produced antibodies in high concentration. By means of precipitin reactions such antiserum could detect minute amounts of thyroglobulin in solution, namely 0.08 to 0.15 mgm. per 100 cc. or $\frac{1}{4}$ to $\frac{1}{2}$ gamma per cent of thyroglobulin iodine. No detectable amounts of thyroglobulin were discovered in the blood of numerous normal patients, 2 myxedema patients, 15 thyrotoxic patients before iodination and 2 thyrotoxic patients after iodination. Similarly no thyroglobulin was detected in the urine of 3 hyperthyroid patients.

It is therefore concluded that the excess iodine usually present in the blood of hyperthyroid patients is not due to circulating thyroglobulin.

Blood obtained directly from the thyroid veins at operation was similarly tested for thyroglobulin. Ten of the twelve samples obtained at the beginning of operation were negative two samples obtained at the end of hemithyroidectomy but from the unoperated side were also negative. On the other hand, seven of the eight thyroid vein samples obtained during or at the end of the second stage of subtotal thyroidectomy showed appreciable amounts of thyroglobulin (0.2 to 13.0 mgm. per 100 cc.) It disappeared from the circulation within 12 to 36 hours after operation.

The presence or absence, or the amount of thyroglobulin in the blood during and after operation, did not correlate with the severity of postoperative reaction.

These results suggest that the presence of thyroglobulin in the blood is due to the extrusion of colloid into the circulation by trauma and that thyroglobulin does not ordinarily leave the follicles as such. This deduction is consistent with observations of Williams (*Am. J. Anat.*, 1937, 62:1) on the release of colloid from living thyroid follicles.

Studies on Hemoglobin Regeneration in Patients with Vitamin C Deficiency By EUGENE L. LOXNER (introduced by George R. Minot) Boston, Mass.

Observations on regeneration of hemoglobin were made in five patients with moderate anemia and vitamin C deficiency as indicated by total absence of reduced ascorbic acid from the blood. These patients were main-

tained on a diet containing only traces of vitamin C and B complex. Three had clinical scurvy, one pellagra, and one "idiopathic" hypochromic anemia. In two patients the degree of anemia was increased by removal of 1600 cc. of blood by venesection. In four of the five patients regeneration of hemoglobin took place spontaneously or in response to iron therapy alone. In one patient, a case of clinical scurvy iron therapy was ineffective but hemoglobin regeneration apparently resulted from the administration of 400 mgm. of ascorbic acid daily. It is concluded that hemoglobin regeneration may occur in the absence of reduced ascorbic acid from the blood by chemical test. It is, however not implied that absence of ascorbic acid from the blood necessarily indicates the total absence of available ascorbic acid.

The Cardiac Failure in Thiamin Deficient Pigeons By ROY LAYER SWANK and OTTO BRASSEY (introduced by Samuel A. Levine) Boston, Mass.

If pigeons are slowly and uniformly depleted of their thiamin on a partially thiamin deficient diet, and starvation prevented by tube feeding they will develop dyspnea in 3 to 6 weeks, and postmortem examination will reveal hydropicardium, pulmonary edema and congestion, congestion of the liver and (or) dependent edema. The myocardia of many of these will show focal necrosis with inflammatory cell infiltration others will appear normal. Thiamin produces rapid recovery in all but the most advanced cases. Electrocardiographic studies reveal two types of abnormality one consisting of changes in the QRS complex in leads one and two, and the other of changes in the T wave in leads two and three. The pathological electrocardiograms are accompanied by a marked increase in the cardiac rate. When thiamin is given, the electrocardiograms and cardiac rate return to normal quickly. A decrease in the cardiac rate occurred only during starvation. It is concluded that a chronic deficiency of thiamin without starvation will produce cardiac failure in the pigeon, that this is preceded and accompanied by pathological electrocardiograms and tachycardia, that starvation alone or during thiamin deficiency produces bradycardia, and that necrosis of the myocardium with inflammatory cell infiltration is a frequent although late finding in these cases.

The Cholesterol Content of the Thoracic Aorta and of the Renal Arteries in Human Necropsy Material: Correlation with Clinical Findings and Vascular Pathology By MAURICE BRUGER and MAURICE R. CHASSIN (introduced by Carl H. Greene) New York, N. Y.

On material obtained from 37 consecutive necropsies, the thoracic aorta and both renal arteries were examined for cholesterol content. The results were correlated with antemortem clinical findings and with vascular pathology. It was observed that (1) The renal arteries of patients who had normal blood pressures during life contained less than 0.9 per cent cholesterol, those of patients with arterial hypertension from 1.0 to 1.9 per cent cholesterol. (2) In subjects who had normal blood pressure the

ratio of aortic cholesterol to renal artery cholesterol was found to increase with age (0.4 in the 1st decade to 3.6 in the 6th decade). This progressive increase in the ratio was disturbed in patients who had hypertension by the increased renal artery cholesterol. (3) The concentration of cholesterol in the renal arteries varied directly with that in the aorta only when the cholesterol content of the latter exceeded 30 per cent. With variations in aortic cholesterol from 0.2 to 30 per cent, low values for renal artery cholesterol were obtained. (4) The degree of renal arteriolar and of coronary artery sclerosis varied directly with the concentration of cholesterol in the renal arteries and in the aorta, respectively.

Mechanism of Hemolysis in Certain Anemias. Significance of Increased Hypotonic Fragility and of Erythrocytosis. By THOMAS HALE HAM* and WILLIAM B. CASTLE, Boston, Mass.

When erythrocytosis and erythro-concentration, normally occurring in the spleen, were imitated *in vitro* by the sterile incubation at body temperature of whole defibrinated mammalian blood, the red blood cells showed progressive increase in volume, in "sphericity" and an increase in hypotonic fragility to such a degree that hemolysis eventually occurred in the serum. These changes were apparently related to metabolic processes and not to hemolytic agents, such as lysolecithin.

Splenomegaly, presumably with increased intravascular stasis, was produced in dogs by prolonged nembutal anesthesia. Blood samples from the splenic vein showed hemoconcentration, variable increase in hypotonic fragility and hemoglobinemia. Concanavalin A of Sumner and Howell, injected intravenously into dogs and rabbits, produced extreme intravascular agglutination, erythrocytosis in peripheral organs and an acute hemolytic anemia which was characterized by a striking increase in "spherocytes," in hypotonic fragility and by hemoglobinemia. Concanavalin A produced no significant hemolysis or change in erythrocyte fragility *in vitro*.

Thus a mechanism is described by which a normal degree of erythrocytosis occurring in the spleen or other organs may cause increased destruction of blood in diseases characterized by increased fragility of erythrocytes, such as congenital hemolytic jaundice, icterus neonatorum and the acute hemolytic anemias caused by arsine and sulfanilamide. Similarly, an abnormal degree of erythrocytosis should produce increased blood destruction in intravascular agglutination in hemolytic transfusion reactions, in hemolytic anemias associated with autoagglutination, in sickle cell anemia, infarcts, and "hypersplenic" anemia.

The Plasma Potassium Rise Accompanying the Paroxysm of Acute Malaria Infections. By R. L. ZWEMER and E. A. H. SIMS (by invitation) and L. T. COGGESHALL, New York, N. Y.

During malaria infections the release of merozoites from the red cells seems to liberate an unidentified toxic

substance into the blood, which is believed to be responsible for the paroxysm and fever occurring at this time. If potassium were released from the parasitized erythrocytes at the time of sporulation, it might serve as a toxic substance. Determinations have been made on the variations in plasma potassium level during the course of experimental malaria infections in monkeys and paretics. There was always a sharp rise in the plasma potassium, but the amount of the increase varied with the strain of parasite used. The sharpness of the rise depended upon the rapidity of the sporulation time. With rapid sporulation it was increasingly difficult to obtain samples at the maximum elevation as its duration was shorter.

Repeated determinations at frequent intervals showed that increments of over 50 per cent may be found. In patients the peak potassium values accompanied chills, preceded the peak temperature, and fell before the drop in temperature.

The potassium release from red cells and its inadequate regulation by the body may be a toxic factor, but it is definitely an accompanying phenomenon of the malaria chill.

Studies on the Effect of Various Pituitary Hormones and Non-Specific Substances Upon the Blood in Hypophysectomized Rats. By OVID O. MEYER and (by invitation) ETHEL W. THEWLIS and HAROLD P. RUSCH, Madison, Wis.

Before this society we reported that hypophysectomy in rats was succeeded by reticulocytopenia and anemia. Exposure of hypophysectomized rats to reduced pressure in a chamber was attended by failure of production of reticulocytosis, polycythemia and bone marrow hyperplasia which normal animals demonstrated. It was further shown that the administration of growth hormone produced marked and prolonged reticulocytosis but no increase in erythrocytes or hemoglobin. The results necessitated further study to establish or exclude the existence of a pituitary hormone with a hemopoietic function. Several workers have concluded that such a hormone does exist but their evidence is not convincing.

It has now been further demonstrated that hypophysectomized rats respond with profound reticulocytosis to the parenteral administration of thyroxine, thyrotropic hormone and adrenotropic hormone. In none of these instances has it been possible to control the post-hypophysectomy anemia. Furthermore, the injection of the sodium salt of cevitic acid (Roche) pH 6.3 to 6.5 or 5 per cent sodium bicarbonate solution is usually attended by slight but significant reticulocytosis.

It is concluded that the reticulocytosis occurring in hypophysectomized rats following the injection of pituitary hormones is non-specific since thyroxine and other substances stimulate their formation. To date, however, no single substance has been found efficacious in producing increases in the hemoglobin or erythrocytes. It is possible that the reticulocytopenia and anemia following hypophysectomy are due to general metabolic derangements. The simplicity of reticulocyte stimulation

* This investigation was aided in part by a grant from the Penrose Fund of the American Philosophical Society.

may be due to their lability in rats, whereas a greater stability of hemoglobin and erythrocyte production may require a more specific stimulus for correction of the defects.

The results tend to indicate the lack of a specific hemopoietic hormone in the anterior hypophysis with a direct action upon the bone marrow.

The Cephalin Cholesterol Flocculation Test As An Aid in the Diagnosis of Hepatic Disorders. By FREDERICK J. POHLE and JOHN K. STEWART (introduced by William S. Middleton) Madison, Wis.

Recent investigations by Hanger (J. Clin. Invest., 1939, 18: 261) indicate that emulsions prepared from sheep brain cephalin and cholesterol are flocculated by serum from patients with hepatic disorders. It was suggested that hepatogenous jaundice could be differentiated from obstructive jaundice by this serological reaction.

In the present study the cephalin-cholesterol flocculation test was performed on serum obtained from 352 normal individuals and 195 patients with suspected intrinsic liver disease. The results were compared with other tests including the icterus index, quantitative determination of the plasma prothrombin, hippuric acid synthesis test, fractionation of the serum proteins and studies on the urobilinogen excretion. Liver tissue obtained from 32 patients at operation or autopsy was examined histologically.

The cephalin-cholesterol flocculation test was negative in all except one of the 352 normal individuals studied. Eighty-eight of the 195 patients showed a flocculation reaction and in each instance clinical or laboratory studies or both confirmed the presence of damage to the liver parenchyma. In 2 patients the test was negative when other evidence indicated that hepatic involvement was present. The degree of flocculation paralleled the severity of the liver disturbance and in patients with hepatitis or cirrhosis of the liver repeated tests proved to be of prognostic significance.

The data indicate that the flocculation test is a more sensitive and accurate index of intrinsic liver disease than any of the so-called liver function tests. In the present study the flocculation test was of little or no value in differentiating obstructive from hepatogenous jaundice. The test was frequently positive in patients with proven biliary obstruction since secondary disturbances in the hepatic parenchyma were also present.

Experimental Production During Rebreathing of Sighing Respiration and Symptoms Resembling Those in Anxiety Attacks in Patients with Anxiety Neurosis. By STANLEY COBB and (by invitation) MANDEL E. COHEN Boston, Mass.

Respiratory and circulatory studies have been made in 100 patients with anxiety neurosis. Anxiety neurosis is a disorder featuring attacks of choking palpitations, trembling and fear. There is general irritability avoidance of crowds and sexual maladjustment.

It was noted, as others have previously stated, that

there is a high incidence of sighing respiration (60 per cent) in these cases as compared with control subjects (10 per cent).

In testing the response of the patients (50 cases) to CO₂ and rebreathing (12 minutes) it was found that the number of sighs was greatly increased. This did not occur as frequently or to as great an extent in the control group. If sighing was not normally present, it did not usually appear as a response to CO₂.

A study was made of patients' feelings (25 cases). During the 12 minute period of rebreathing most of these stated that they experienced feelings and sensations resembling or identical with their anxiety attacks. In some cases the patient exhibited observable differences in behavior during rebreathing such as tearing off mouth-piece, clutching at throat writhing and wringing hands.

Studies Pertaining to the Metabolism of Synovial Fluid Mucin. By MARIAN W. ROPES and WILLIAM V.B. ROBERTSON (by invitation) and WALTER BAUER, Boston, Mass.

Synovial fluid mucin is an easily dissociable protein polysaccharide complex of remarkably constant composition and characteristics. Physically and enzymatically it is similar to the mucin of subcutaneous tissue. Therefore, the metabolism of mucin is important in the physiology not only of joints but also of connective tissue in general.

The modes of formation and destruction of mucin are not definitely known. Evidence to date indicates that it is formed by synovial tissue cells and carried into the joint by the plasma dialysate which forms the synovial fluid.

The difficulty in removal of globulins, which are smaller molecules than mucin, from normal joints and the altered characteristics of mucin in pathological effusions, suggest that mucin is broken down prior to removal. Additional evidence has been obtained from studies of the glucosamine content of normal fluid and pathological effusions before and after precipitation of mucin.

Our studies have disclosed three types of agents which may play a rôle in the destruction of mucin and its polysaccharide—bacterial enzymes phosphatase and ascorbic acid peroxidase.

Mucinase, an enzyme obtained from filtered cultures of *Cl. perfringens* and various other bacteria, depolymerizes mucin and partially hydrolyzes the polysaccharide. Attempts to demonstrate the presence of mucinase in normal joint fluid or in pathological effusions in which mucin had been partially destroyed have been unsuccessful.

Phosphatase also causes breakdown of synovial fluid mucin. The marked and unexplained variations in the concentration of phosphatase in joint fluid suggest that it may be one factor in the metabolism of mucin.

More pertinent physiologically is the destruction of mucin by vitamin C. We have shown that ascorbic acid-peroxidase, an oxidation product intermediate between ascorbic acid and dehydroascorbic acid, causes depoly-

merization of mucin or its polysaccharide. Here, as in the case of the destruction of mucin in joint disease, glucosamine is not liberated. The action of ascorbic acid-peroxide is not limited to mucins of mesothelial origin but depolymerizes also epithelial mucins, starch, pectin, chondroitin sulfuric acid from cartilage and capsular polysaccharides of pneumococcus

The possibility that ascorbic acid plays a rôle in the metabolism of mucin is supported by the abnormalities of vitamin C metabolism in rheumatoid arthritis. It has been shown that the level of vitamin C in the blood is reduced, and often can be raised to normal only by the administration of massive doses of ascorbic acid. Metabolic experiments in this laboratory indicate that a large part of the ingested ascorbic acid does not remain in the reduced form in patients with rheumatoid arthritis. We have, however, found no difference in the power of normal and rheumatoid serum or synovial fluid to oxidize ascorbic acid.

The depolymerizing activity of ascorbic acid-peroxide on synovial fluid and other connective tissue mucins, the abnormalities of vitamin C metabolism in rheumatoid arthritis and the marked involvement of mucin-containing tissues in this disease suggest that ascorbic acid plays a role in the mechanism of tissue changes in rheumatoid arthritis but in no way indicate an etiological relationship

The Bactericidal Property of Blood in Gonococcal Infections By HOWARD C. COGGESHALL, HELEN B. ARNOLD and L. DIENES (introduced by Charles L. Short), Boston, Mass

Various investigators have used the bactericidal property of blood to measure immunological response. Recently, it has been employed in gonococcal infections, but the results are not conclusive because of the lack of control data. For evaluation of this test we selected 44 cases with uncomplicated and complicated gonococcal infections (11 acute urethritis, 9 prostatitis, 10 salpingitis, 2 epididymitis and 12 arthritis). Fifty-three tests were made on these cases and were controlled by 150 tests on 43 normals. Gonococcal complement fixation was determined on all bloods.

The method was similar to that employed by Todd, Ward, Robertson and Keefer. Most tests were made with a strain isolated from an acute case of urethritis. However, a total of 13 strains isolated from other sources was tested.

Blood obtained from controls exerted in most instances a marked bactericidal effect which varied from 0 to 1,000,000 organisms killed, the median being 10,000. Uncomplicated cases of gonorrhea showed the same range and distribution of blood bactericidal activity. However, complicated cases showed a slight increase in the average bactericidal titer, the median being 100,000. Results from any given case were not significant because of the wide variations observed in both normal and infected cases.

Comparison of 13 strains of gonococci showed definite variations in resistance to the bactericidal activity of blood. Strains resistant to normal blood tended to be

equally resistant to blood obtained from the patients studied. Since the bactericidal property of blood from patients with gonorrhea or one of its complications is not increased strongly and regularly, even after the gonococcal fixation test becomes positive and does not differ significantly from that of controls, it would seem that this test in its present form is not an adequate measure of the immunological response to gonococcal infections.

The Passage of Thiocyanate and Glucose from the Blood Stream into the Joint Spaces By J. WALLACE ZELLER and E. G. L. BYWATERS (introduced by Granville A. Bennett), Boston, Mass

In order to obtain further information concerning the physiology of articular structures, the permeability of the synovial tissues to the crystalloids, NaCNS, and glucose was studied in normal calves. Thiocyanate was detectable in the synovial fluid nine minutes after intravenous injection. Fairly constant serum CNS levels were reached within one hour, but the time required for attaining equilibrium between the serum and the synovial fluid varied from one to six hours. At equilibrium the CNS in serum averaged 9 per cent higher than in synovial fluid. The CNS content of aqueous humor was about one-third that of synovial fluid, and only traces were present in the cistern fluid. The CNS concentrations in these three body fluids and in the serum roughly paralleled the protein concentrations. A similar relationship between CNS and protein concentrations of serum and transudates has been observed in patients with edema. Our results confirm the suggestion that some of the CNS is held in the serum in a non-diffusible state.

Glucose entered the joint space more slowly, appearing about twenty minutes after intravenous injection. The blood sugar returned to the pre-injection level in approximately two hours. When the blood sugar was falling, the rate of utilization of glucose by the articular tissues and the rate of diffusion from the joint space into the synovial capillaries were not sufficiently rapid to keep the synovial fluid sugar level at or below that of the serum. Similar relationships between blood and pleural transudates in diabetic patients with cardiac failure have been described.

The experiments show that substances of small molecular size diffuse readily into the joint spaces. With respect to CNS and glucose, the equilibrium between serum and normal synovial fluid resembles the equilibrium between serum and transudates in patients with edema. This similarity offers further indication that synovial fluid is tissue fluid.

The Treatment of Hypogonadism By W. O. THOMPSON and (by invitation) N. J. HECKEL and S. G. TAYLOR, III, Chicago, Ill

The following aspects of the treatment of hypogonadism are of special interest

1 *Factors limiting response to glandular treatment*
The response to treatment is determined by the stimulat-

ing agent and the capacity of the organs stimulated to respond. Striking growth of the genitalia, other secondary sexual characteristics, and the skeleton may be induced by some gonadotropic materials and sex hormones, but the response appears to be modified greatly by the age at which the stimulus is applied. For example, at any age from birth to puberty the penis may be made to grow to adult proportions (but not larger) by administration of the gonadotropic material from the urine of pregnant women. After puberty striking growth may be induced only if the penis is very small but the growth never appears to equal the normal. The growth of body hair roughly parallels that of the penis. A similar state of affairs obtains in the treatment of young boys and of adults of the eunuch and eunuchoid types with male sex hormone (testosterone propionate). The response of the genitalia and other secondary sexual characteristics in the female with primary hypogonadism to treatment with estrogenic materials, including stilboestrol bears a similar relationship to the age of the patient.

There appears to be an optimum time for induction of genital growth and the changes associated with it and this time is apparently the period during which they normally occur.

2. *Hormonal factors influencing the growth of the prostate in man* The following observations in man support the hypothesis that the growth of the prostate is dependent upon the production of male sex hormone.

(a) In young boys showing marked genital growth during the administration of gonadotropic principles or male sex hormone, and in adult eunuch and eunuchoid individuals showing similar changes during the administration of male sex hormone, the prostate which is commonly not palpable before treatment may assume the proportions seen in the normal adult.

(b) The prostate regresses in size when such treatment is omitted.

(c) The prostate normally enlarges at the time of puberty in association with a marked increase in the production of male sex hormone.

These observations would appear to contraindicate the treatment of benign prostatic hypertrophy with testosterone propionate.

In elderly men with waning sexual function, who experience marked improvement during the administration of testosterone propionate, the size of the prostate should be checked frequently.

The Destruction of Thiamin by Unacidified Bile and Pancreatic Juice A Possible Explanation of the Cord Changes in Pernicious Anemia. By HENRY FIELD, JR. and (by invitation) WILLIAM D. ROBINSON and DANIEL MELNICK, Ann Arbor Mich.

Degenerative changes in the spinal cord, more or less simulating those of pernicious anemia, have been observed in beriberi, pellagra and experimental deficiencies of vitamins A, B and some unidentified factor in the B₁₂ complex. A deficiency in intake of these vitamins in

pernicious anemia has not been demonstrated but a deficiency might be conditioned by the pathological physiology of the disease.

We have found that patients receiving intensive alkali therapy for peptic ulcer and those with achlorhydria have subnormal urinary excretions of thiamin. There is no significant destruction of thiamin when it is incubated with achlorhydric gastric juice. As much as 56 per cent of thiamin is destroyed when it is incubated with human bile or pancreatic juice at their natural pH. A relatively small destruction occurs when thiamin is incubated with bile or pancreatic juice adjusted to the pH commonly found in the intestinal tract.

The patient with achlorhydria will develop a thiamin deficiency unless he takes in more of it than will protect a normal individual. A chronic, variable thiamin deficiency over many years may explain the cord changes of pernicious anemia. It is possible that the abnormal physiology of this disease may effect the utilization of other vitamins.

Chloride Excretion in Experimental Diabetes Insipidus

By E. HENRY KEUTMANN and ROWLAND T. BELLOWES (introduced by S. L. Warren) Rochester N. Y.

Chloride and water excretions were studied in dogs with diabetes insipidus. The disease was produced by cauterization of the supra-optic nuclei. The rate of glomerular filtration was calculated by means of creatinine clearances.

The response to the intravenous injection of various concentrations of salt solution was studied. Before operation the maximum ratio of $\frac{\text{Cl. in urine}}{\text{Cl. in serum}}$ obtained when 5 per cent sodium chloride solution was injected was 2.36. After operation the maximum of this ratio obtained with identical procedure was 0.59. The clearance of chloride was also reduced.

Similar differences before and after operation were found when less concentrated salt solutions were used.

When pitressin was administered to animals with diabetes insipidus, there was an immediate and marked increase of the ratio $\frac{\text{Cl. in urine}}{\text{Cl. in serum}}$ and an immediate increase in the clearance of chloride.

After operation the rate of glomerular filtration decreased. When pitressin was administered, there was no immediate increase of glomerular filtration while the chloride concentration and excretion increased. It is thought that the changes in chloride excretion can best be explained by increased reabsorption of chloride after the production of diabetes insipidus.

Observations on Resistance of Pneumococci to Sulfapyridine and Sulfathiazole By FRANCIS C. LOWELL and ELIAS STRAUSS (by invitation) and MAXWELL FINLAND, Boston, Mass.

The susceptibility of different strains of pneumococci to the action of these drugs was found to be considerable variations. These variations in

were observed in different laboratory strains, in strains isolated from different patients and in strains obtained from the same patient at different times in the course of treatment.

Susceptible strains were made resistant by various *in vitro* procedures. Different strains varied in the ease with which they acquired resistance.

When strains that were originally susceptible to the action of both sulfapyridine and sulfathiazole were made resistant to the action of either one of these chemicals they also acquired resistance to the other.

The Positive Conditional Salivary Reflexes in Psychoneurotic Patients By GEORGE F. SUTHERLAND (by invitation), JACOB E. FINESINGER and (by invitation) FRANCIS MCGUIRE, Boston, Mass.

This study deals with variations found in the positive salivary reflex in eighteen psychoneurotic patients.

1 The magnitude of the response varied from day to day, even from combination to combination.

2 The greatest response usually occurred in the second combination of the day.

3 A metronome stimulus elicited greater response than did a 100-watt light stimulus.

4 A correlation existed between the emotional state, as determined by an interview, and the reflex status. Some patients developed progressive inhibitory states characterized by (1) a diminution in response, (2) equalization of response to auditory and visual stimuli, (3) paradoxical reaction, (4) ultra-paradoxical reaction, and (5) temporary inhibition of the reflex.

The Occurrence of Methemoglobinemia During Therapy with Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole. Formation of Methemoglobin in Vitro By CHARLES L. FOX, JR. and BRUCE HOGG (by invitation) and REUBEN OTTENBERG, New York, N. Y.

The blood of patients receiving sulfanilamide has been shown to contain methemoglobin and an additional pigment. Patients receiving sulfapyridine, sulfathiazole, and sulfamethylthiazole do not appear as deeply cyanotic as patients receiving sulfanilamide. Like sulfanilamide, these three drugs do not produce methemoglobin *in vitro*. Photo-oxidized sulfanilamide has, however, been shown to produce methemoglobin *in vitro*. In seeking to explain the mechanism of anti-bacterial action of these drugs, it seemed important to ascertain whether methemoglobinemia accompanies therapy and, if possible, to reproduce this *in vitro*.

Curves drawn by the Hardy recording spectrophotometer, and the change in optical density at λ 630 m μ after addition of cyanide, clearly demonstrated methemoglobinemia during therapy with these three drugs.

In vitro, no methemoglobin was formed by any of the drugs. After oxidation of these drugs by irradiation with ultraviolet light, the resulting faintly yellow solutions all produced methemoglobin.

Complete absorption curves were also obtained on mixtures of hemoglobin and solutions of the photo-oxidized

drugs. As in the case of sulfanilamide, analysis of these curves and those of patients' blood demonstrated, in addition to the methemoglobin produced, a third unidentified pigment with maximum absorption in the red end of the spectrum.

The data show that therapy with these drugs is accompanied by methemoglobinemia. The *in vitro* formation of methemoglobin only by oxidized forms of these drugs suggests that the methemoglobinemia occurring during therapy results from oxidation of the drugs used.

Studies of Homogentisic Acid Production in a Case of Alkaptonuria By J. MURRAY STEELE and (by invitation) KONRAD DOBRINER and MORTON GILDSTON, New York, N. Y.

The study concerns the effect of feeding various amounts of protein and certain vitamins and of injecting *D*- and *L*-phenylalanine upon homogentisic acid production in a 56-year-old man.

On a protein diet averaging 130 grams daily he excreted 78 grams of homogentisic acid in 24 hours, on a 60 gram diet, 36 grams, and on a 30 gram diet, 18 grams of homogentisic acid. On the high protein diet neither daily injections of crude liver extract nor campolon had any effect within a period of 6 days. Nicotinic acid, thiamine chloride, riboflavin and other vitamin studies are under way. Nicotinic acid in 1 gram doses daily is without effect upon the homogentisic acid excretion.

After 7 days' observation on the low protein diet, 4 grams of *D*-phenylalanine were injected intravenously. During this 24-hour period the homogentisic acid excreted rose from an average of 18 to 34 grams, and the urinary carboxyl carbon from 47.4 to 129.3 mgm. Four days later 4 grams of *L*-phenylalanine were injected and the homogentisic acid excreted rose from 18 grams to 56 grams while the urinary carboxyl carbon did not rise significantly. Of the *D*-phenylalanine injected only 40 per cent was converted into homogentisic acid, while of the *L*-phenylalanine, 95 per cent was converted. If the rise in urinary carboxyl carbon is calculated as phenylalanine, then of the *D*-phenylalanine injected, 28 per cent was excreted as amino acid, while of the *L*-phenylalanine, there was none or only a trace.

Pregnancy in Relation to Rheumatic Fever and Rheumatic Heart Disease By BERNARD J. WALSH (by invitation) and EDWARD F. BLAND and T. DUCKETT JONES, Boston, Mass.

The continued follow-up of a large number of patients who have received care at the House of the Good Samaritan because of rheumatic fever has provided data pertaining to pregnancy and its influence on this group. These data are of particular interest because they include observations made for considerable periods both before and after pregnancy.

The material consists of 264 births occurring in 153 women, or 1.7 living children per patient. An additional 34 births took place prior to the seventh month of pregnancy. The mean age for the group at their initial

pregnancy was $21\frac{1}{2}$ years. Rheumatic heart disease was present in 60 per cent.

In only 13 instances (5 per cent of the total live births) did events occur in relation to pregnancy that might be considered as evidence of active rheumatic fever. These events may be divided into three groups:

1. Frank rheumatic fever with joint pain and swelling 4 patients.
2. Development of congestive failure during the eighth and ninth months of pregnancy 5 instances in 4 patients.
3. The development of congestive failure after pregnancy 4 patients.

In ascribing the congestive failure that appeared in

those of Groups 2 and 3 to rheumatic fever we affirm our belief that the occurrence of congestive failure in adolescent or young adult patients with rheumatic heart disease is by and large an indication of active rheumatic fever. However cardiac failure becoming manifest during the last trimester of pregnancy renders this likely rôle of active rheumatic fever a more uncertain one. Conversely the development of congestive failure in the postpartum period, a time when blood volume, blood flow and the cardiac output have returned to normal, makes active rheumatic fever a likely causative agent in the production of the congestive failure.

Excepting the cases previously mentioned, there has been no detectable progress in cardiac disease that could be related to pregnancy.

edema Pescatori (8) studied 40 postmortem fluids and found an average pH of 7.95 with variations from 7.53 to 8.02

Normal human synovial fluid contains fewer cells than normal bovine fluid, the average nucleated cell count being 63 per cu mm (3) as compared to 112 to 182 (1, 9, 10). Erythrocytes are absent. The average differential nucleated cell counts for normal human and bovine synovial fluid are as follows

	Human fluid	Bovine fluid
	<i>per cent</i>	
Polymorphonuclears	65	22
Monocytes	47.9	36.4
Clasmatocytes	10.1	15.0
Unclassified phagocytes	4.9	3.9
Lymphocytes	24.6	40.1
Synovial cells	4.3	1.2
Unidentified cells	2.2	1.2

The total nucleated cell counts of this series can be compared with those reported for normal human fluids by Labor and von Balogh (11) (10 to 20 cells per cu mm), McEwen (12) (125 to 200 cells), and Kling (13) (10 to 50 cells)

The total protein content of normal human fluid (2.57 grams per 100 cc) is approximately three times as high as that of normal cattle fluid. Since most of the increase is in the mucin fraction, the content of albumin and globulin is only twice as high in human fluid, being 1.72 grams as compared to 0.89 gram per 100 cc. This value is in the same range as those reported by Fisher (5) (1.6 per cent) and Horiye (4) (0.45 to 3.15 per cent) for normal human fluid. On the basis of the findings in many normal and abnormal human fluids, we would conclude that a value of 3.15 would be found only in an abnormal fluid. Cajori and Pemberton (14) reported 1.39 per cent protein in synovial fluid from a patient with generalized edema. Determination of the albumin and globulin fractions of human fluid was possible only when 2 cc of fluid was obtained, with the result that the majority of determinations were made on fluids aspirated from edematous patients. Furthermore, the uncertainty of the absolute globulin concentration by any of the methods for fractionating the serum proteins, and the decreased accuracy of the methods in fluids of low protein content, make it difficult to establish accurate normal values for the protein fractions. The marked individual variations that were found

may be due in part to analytical difficulties and in part to variation in capillary permeability. Similar variations have been found in normal cattle fluid (1) and in pathological fluids (14). In all cases, however, the globulin content was low and in one edema fluid no globulin was demonstrable. The albumin-globulin ratios tended to be high. In the one case in which only 2 cc of fluid was obtained the ratio was 19.3, with a globulin concentration of 0.05 gram per 100 cc.

The presence of albumin and globulin in normal human fluid can be explained presumably on the basis of slight capillary permeability to proteins, as was assumed in the case of normal cattle fluid. The marked difference in albumin and globulin concentration indicates a much greater capillary permeability to albumin than to globulin. This is in accord with the findings in cattle fluid (1), with the results of the studies on the entrance of proteins into joints of rabbits (15), and with the conclusions of other workers (16, 17, 18, 19).

The mucin content of normal human fluid (0.85 gram per 100 cc) is much higher than that of fluid from the astragalotibial joints of cattle, as would be expected from the marked differences in viscosity. Fisher (5) found 1.95 per cent mucin in normal human fluid, while Cajori and Pemberton (14) report a mucin content of 0.42 per cent in fluid from a patient with generalized edema. That the viscosity of synovial fluid is due to the presence of mucin is shown by the fact that the viscosity approaches that of water after removal of the mucin. Comparison of the differences in viscosity and mucin in normal synovial fluids from various animals suggests that the viscosity is related to the degree of polymerization of mucin as well as to the concentration.

Normal human synovial fluid does not clot. This is due presumably to the absence of fibrinogen. No fibrinogen was found by precipitation experiments with 1.1 M phosphate solution at pH 6.5.

The distribution of nonprotein nitrogen between fluid and plasma is approximately the same as that found in cattle fluid (1), in horse fluid (20), and in other fluids shown to have the composition of dialysates of blood plasma (21, 22, 23). The average distribution ratio is 0.91, and many individual cases show an equal concen-

TABLE I
Chemical composition of normal human synovial fluid

	Amount	Relative viscosity	Total solids	Specific gravity	pH	Total protein (exclusive of mucin)		Albumin	Globulin	Mucin
						Fluid	Serum			
	cc.		grams per 100 grams			grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.	grams per 100 cc.
Average *	1.1	150	3.41		7.38	1.72	6.79	1.02	0.05	0.85
Maximum	3.5	403	4.83		7.39	2.13	7.26			1.10
Minimum	0.13	51	2.40		7.36	1.31	6.31			0.55
Number of fluids †	46	20	8		2	10	2	1	1	5
Average ‡	8.0	53	2.23	1.010	7.40	0.63	5.77	0.67	0.17	0.50
Maximum	40.0	174	3.43	1.012	7.45	1.78	7.08	1.45	0.33	1.18
Minimum	0.25	6	1.45	1.008	7.29	0.12	4.44	0.12	0	0.06
Number of fluids ‡	78	7	19	7	4	60	24	6	6	48

* Fluids obtained postmortem from patients without edema.

† Fluids obtained postmortem from patients with varying degrees of edema

‡ Represents the number of fluids from which the averages were obtained.

tration in plasma and fluid, indicating that the tissue between fluid and plasma in humans is readily permeable to nonprotein nitrogen

The distribution ratios for sugar show more variation, but the average is 1.12 and many cases show a ratio of 1.00. The variations and the high average ratio are explicable by the fact that the blood sugar was changing rapidly before death and the fluid was not in equilibrium

Study of the distribution of electrolytes between fluid and plasma in humans has been limited because of the small amount of fluid available. Such determinations as we have made represent fluid from patients with edema (see Table II)

TABLE II
Distribution ratios between serum and synovial fluid

	NPN/ NPN	Sugar/ Sugar	Cl Cl _r	$\frac{\sqrt{Ca_r}}{\sqrt{Ca_s}}$
Average	0.91	1.12	0.98	0.87
Maximum	1.11	2.06	1.00	0.90
Minimum	0.66	0.65	0.91	0.84
Number of fluids*	25	22	15	4

* Represents the number of fluids from which the averages were obtained.

The results, however, are applicable to normal human synovial fluid in general, since membrane equilibrium, if present, would be maintained despite the increased volume of fluid. The two substances (Cl and Ca) which have been studied show distributions similar to those found in cattle fluid and other body fluids having the composition

of plasma dialysates. The average distribution ratio of chloride between plasma and human fluid is identical with that for cattle fluid (1). The theoretical Donnan ratio for the two systems is

the same. The average ratio $\sqrt{\frac{Ca_r}{Ca_s}}$ also is essen-

tially the same as that for cattle fluid and indicates that part of the calcium is held in the serum presumably bound to protein.

DISCUSSION

Thus, the distribution of non-electrolytes and electrolytes between plasma and normal human synovial fluid is in accord with the findings in normal bovine synovial fluid and indicates that normal human synovial fluid is a dialysate of blood plasma, containing albumin globulin and mucin.

The presence of serum proteins in synovial fluid can be explained presumably on the basis of slight capillary permeability. Albumin and globulin are found in varying amounts in other body fluids which have been shown to have the composition of simple dialysates of blood plasma (lymph, edema pleural and ascitic fluids) (21, 22, 23). The high albumin globulin ratio in normal human fluid indicates a greater permeability to albumin than to globulin.

The presence of mucin in no way invalidates the above theory. The nature of the synovial membrane and the mechanism of formation of mucin have long been discussed. The consensus

EXCRETION OF SODIUM PREGNANEDIOL GLUCURONIDATE IN URINE OF NORMAL HUMAN PREGNANCY¹

By CARL BACHMAN DOROTHY LEEKLEY, AND H. HIRSCHMANN

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(Received for publication June 17 1940)

Venning and Browne (1 to 7) have shown that sodium pregnanediol glucuronide is excreted in urine of human pregnancy in concentrations which become increasingly higher as gestation approaches term. Although this demonstration has been repeatedly confirmed (8 to 16) relatively few workers have studied the precise levels and ranges of normal excretion, or have secured long-continued records of output in individual parturients. As a consequence there has not been complete agreement concerning the quantitative aspects of normal excretion. We have accordingly studied the urines of a number of healthy women throughout pregnancy. In addition to corroborating the original findings of Venning and Browne, our studies have thrown light upon a number of points which have previously been in question, and have supplied data concerning excretion during multiple pregnancy.

METHODS

Twenty four hour volumes of urine were collected at weekly intervals from early pregnancy to the onset of labor. The specimens were delivered to the laboratory within an hour of the completion of their collection, and were then kept at 0 to 5 until their work-up, which in no instance was delayed longer than 15 hours.

The work up of the urines was carried out in accordance with Venning's modified method (3) except that 3.0 cc. of water were used for re-dissolving the first precipitates obtained from acetone. From the 24-hour collections, aliquots were selected which were expected to yield at least 15 mgm. of the glucuronide. When the pure compound was added to pregnancy urines in this amount, recoveries were quantitative with the procedure employed. This did not justify the assumption that the recoveries of the pre-formed glucuronide of such urines were also quantitative, since the addition of 15 mgm. of pure compound to urines containing no glucuronide—i.e. male or menopausal urine—resulted in recoveries of only about 85 per cent. Nevertheless, the yields of pre-formed compound from pregnancy urine were calculated

on the assumption that they represented complete recoveries.

The pure reference compound was obtained by extracting the final product of the modified Venning procedure with butanol from an alkaline solution, and then evaporating the extract to dryness, precipitating the residue from water with acetone and recrystallizing the product from ethanol. Examination of a sample, dried at room temperature *in vacuo* over P_2O_5 , gave the following analysis²

Calculated for $C_{27}H_{46}O_6Na$, H_2O	C, 60.41	H, 8.45	Na, 4.29
$C_{27}H_{46}O_6Na$	C, 62.51	H, 8.36	Na, 4.44
Found	C, 62.35	H, 8.21	Na, 4.63

These findings together with our data on the loss of weight during drying indicated that the pure compound did not contain a molecule of water of crystallization, as stated by Venning and Browne (1) and that a monohydrate could not be readily obtained. Its melting point was found to vary with the rate at which samples were heated, being 277 (corrected) when the temperature was raised 4 per minute. Melting was accompanied by decomposition.

The pregnanediol glucuronide isolated during the systematic examinations of pregnancy urine was identified in every instance by a melting point determination, standardized to the above rate of heating. In general, the preparations obtained from urine of early and mid-pregnancy were relatively colorless crystals which rarely melted at temperatures lower than 273 (corrected). Those obtained from urine of late pregnancy were brown and semi-crystalline, and showed melting points 6 to 10 lower than that of the standard preparation. Although it was obvious that the impurities present in such samples caused over-estimates of the amounts of glucuronide obtained, no correction was applied to the yields. However preparations which gave melting points more than 10 lower than the standard were further purified before weighing.

In conformity with previous reports, our results have been recorded in terms of free pregnanediol. Since our glucuronide products were dried *in vacuo* before weighing, a factor based upon the molecular weight of anhydrous pregnanediol glucuronide—i.e., 320/518, or 0.618—was employed for this conversion.

OBSERVATIONS

Single fetus gestation Data were obtained from 6 patients whose health remained normal

¹Added by a grant from the Penrose Fund of the American Philosophical Society

²Microanalysis by Mr. Wm. Saschek.

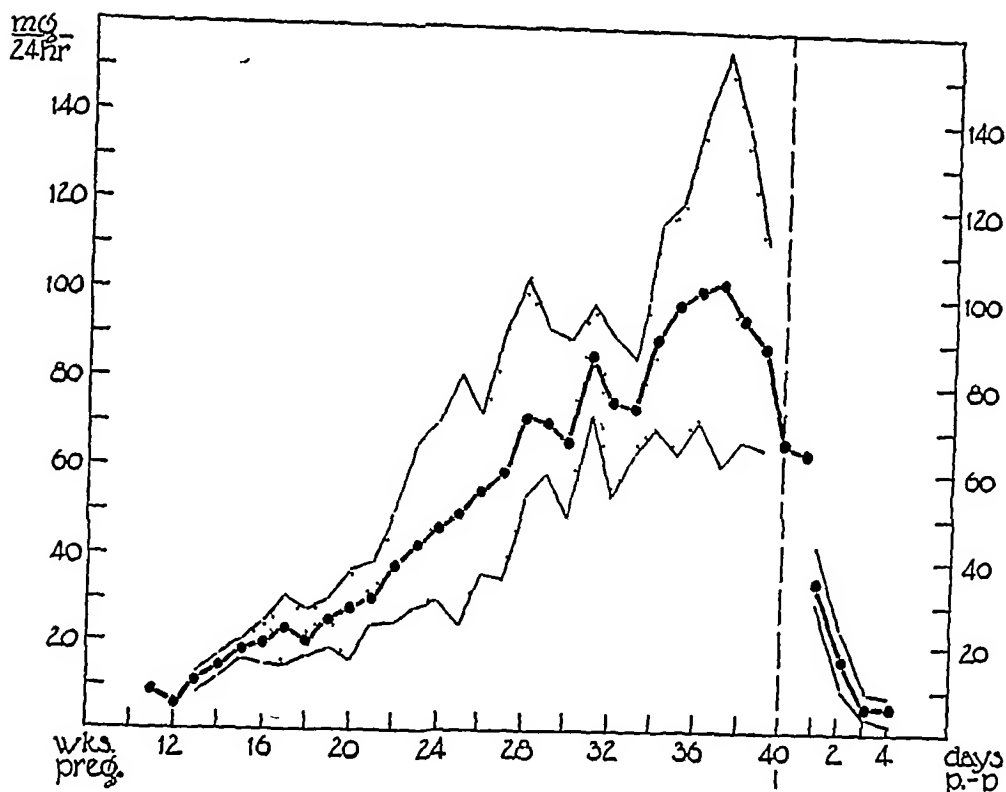


FIG 1 PREGNANEDIOL EXCRETION IN NORMAL PREGNANCY

Abscissa represents menstrual age of pregnancy in weeks (wks preg), and includes the first few postpartal days (days p-p). Ordinate shows output of pregnanediol in mgm. per 24 hours. Heavy dotted line represents the means, light lines (enclosing stippled area) the extremes, of daily excretion for various weeks of gestation in a group of 6 women. All labors spontaneous in onset. Average duration of pregnancy for the group, 279 days. Average crown-heel length of infants, 49.2 cm, average weight, 3218 grams. Average placental weight, 630 grams.

during the period of study. The results of 144 determinations were available for consideration, representing an average of 24 serial weekly examinations of each patient prior to the onset of labor. It was noted that with each patient these examinations had fallen upon dates which could be oriented in terms of weekly intervals (plus or minus a day) from either the date of the last menstruation or the date of delivery. It was thus possible to arrange the data in terms of the mean and extreme excretions of the group for each week of gestation.

Such an arrangement of the data has been made in Figures 1 and 2, where the orientations have been based, respectively, upon the menstrual age of the pregnancies and upon the number of weeks remaining before delivery. That the two methods of orienting the data should not have yielded identical curves of average excretion was due to the fact that the menstrual age of the pregnancy at

the time of delivery in 3 of the 6 patients deviated from the mean of 40 weeks for human pregnancy by 1 to 2 weeks, *i.e.*, 38, 39 and 42 weeks, respectively. The method of orientation and the resultant curves illustrated in Figure 2 have been regarded as preferable to those of Figure 1 for the following reasons. First, the menstrual histories were not completely dependable in certain of the cases, second, the infants who were born to each of these mothers met the accepted criteria for full fetal maturity in respect to their skeletal dimensions and body weights regardless of the presumptive menstrual ages of the gestations, third, the deviations of the extreme values from the mean values of excretion were smaller in Figure 2 than in Figure 1, and, finally, Figure 2 reflected certain characteristics of the patients' individual records of excretion more fully than did Figure 1.

Prior to the 20th week of pregnancy mean

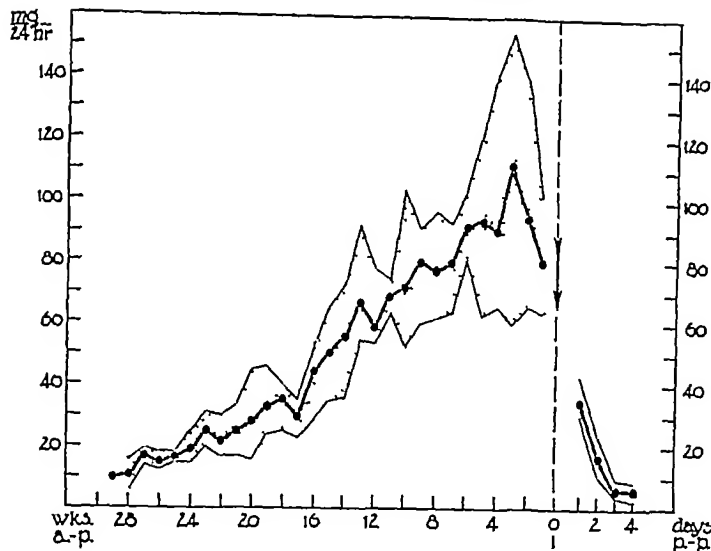


FIG. 2. PREGNANEDIOL EXCRETION IN NORMAL PREGNANCY

Same as Figure 1 except that the data for various periods of pregnancy are arranged in terms of intervals remaining before labor or weeks antepartum (wks. a. p.)

daily excretions of pregnanediol did not exceed 30 mgm. Thereafter the amounts of such excretions were found to rise by increments of about 20 mgm per month until the 36th week, at which time a peak level of about 100 mgm. per 24 hours was attained. Following this the daily excretions tended to fall off in amount, so that by the 39th week a mean level of 80 mgm per day was the rule. According to Figure 2 the amount of any patient's daily excretion did not appear to deviate by more than ± 20 per cent from the mean excretion of the group for a corresponding period. Among the various patients these differences tended to be most marked in late pregnancy. No patient's individual record of output was found to lie continuously above or below the mean curve for the group.

The pre-labor peak and fall in excretion, illustrated in Figures 1 and 2, were apparent in the individual excretion records of 5 of the 6 patients studied. In addition transient cyclic fluctuations, recurring at monthly intervals, were clearly visible in the records of 4 of these patients (see also the

averaged data of Figure 2). These fluctuations were not paralleled by similar variations in the urine volume output. Moreover, an investigation of the latter point illustrated in Table I showed that when urine volume and pregnanediol output

TABLE I

Absence of correlation between urine volume output and excretion of pregnanediol glucuronide during short consecutive intervals

Case number	Days antepartum	Collection period	Urinary volume	Pregnanediol glucuronide
		hours	cc.	mgm.
1	98	6	1100	20.8
		6	620	15.4
		6	420	24.0
		6	375	22.8
2	31	6	230	30.1
		6	500	26.9
		6	350	32.6
		6	575	31.2
3	1	6	160	46.2
		6	185	37.8
		6	160	19.4
		6	140	14.3

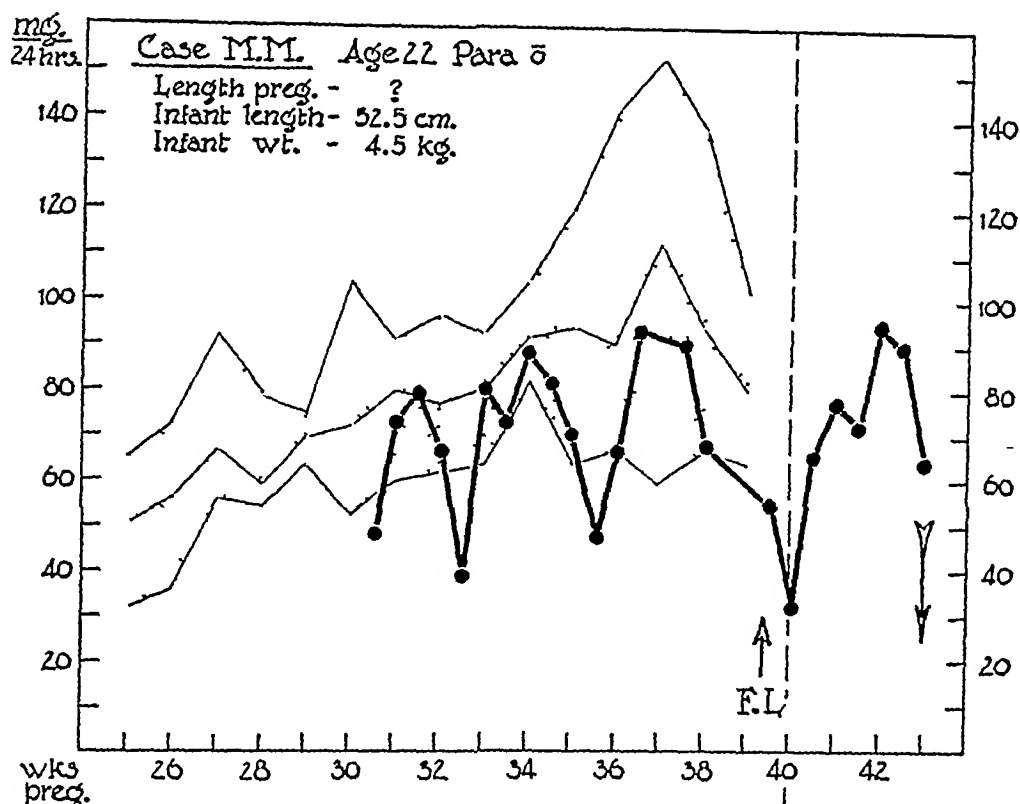


FIG 3 CYCLIC FLUCTUATIONS OF PREGNANEDIOL EXCRETION DURING LATE PREGNANCY IN A PRIMIGRAVIDA

Excretion at twice-weekly intervals (heavy dotted line) is plotted against a background (stippled area) of the normal ranges of pregnanediol output for the period studied. False labor (F.L.) occurred at 40th week. True labor, and the birth of an oversize postmature infant, occurred at 43d week.

were both examined during short consecutive intervals, there was no general relation between them either at periods of pregnancy, when the rate of pregnanediol excretion was relatively constant,³ or immediately prior to labor when the rate was falling precipitously.

In order to verify the existence of these cyclic fluctuations in the excretion of pregnanediol, the urine of a seventh patient was examined at twice-weekly intervals during the last 3 months of gestation. The results, plotted in Figure 3, fully confirmed those obtained in the original group of patients.

Multiple pregnancy Results were obtained for short periods of gestation from 4 cases of twin, and 1 case of triplet pregnancy. They have been

plotted in Figure 4, together with data concerning the dimensions and weights of the infants and placentas. Information concerning the maternal health of these cases has been included (see Table II) in order that this factor could be considered in evaluating the probable physiologic significance of the results. With the exception of the inexplicably high level of excretion exhibited during early pregnancy in one case, the results indicated that pregnanediol output in these cases of multiple pregnancy did not exceed the levels observed at corresponding periods of single-fetus gestation.

Puerperium Results covering the first 5 days of the puerperium were obtained from 5 patients whose excretions were normal during pregnancy. In spite of the use of an in-dwelling catheter for obtaining the urine free of contamination with lochial discharges, it was found that the glucuronide isolated from puerperal urine was more

³ In view of this finding we are currently investigating the possibilities of substituting 12-hour specimens for 24-hour collections of urine in the study of pregnanediol excretion in pregnancy.

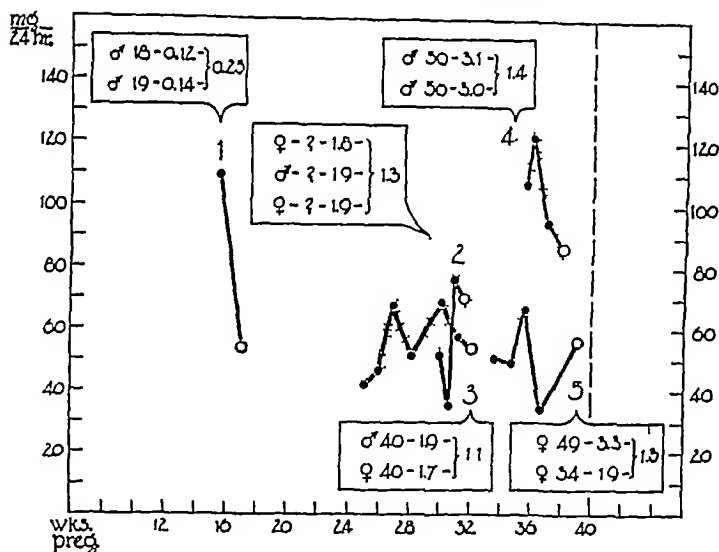


FIG. 4 PREGNANEDIOL EXCRETION IN MULTIPLE PREGNANCY

Data for short periods of gestation in 4 cases of twin, and 1 case of triplet pregnancy are plotted against a background (stippled area) of normal ranges of pregnanediol excretion for single-fetus gestation. Each heavy dotted line represents excretion in an individual patient, and is identified by a case number by the use of these numbers the clinical data concerning each patient may be located in Table II. The symbols and numerals enclosed within the brackets represent respectively the sex, crown-heel length (in cm.) and weight (in kgm.) of the infants and the weight (in kgm.) of the combined placentas for each patient.

impure than that obtained from the same patients during pregnancy. In each instance it was necessary to purify this product before precipitates possessing satisfactory melting points could be obtained for weighing. The results recorded in Figures 1 and 2 showed a fair degree of constancy among the 5 patients. About 30 mgm of pregnanediol, on the average, were excreted during the 24 hours following delivery. Material of low

melting point, averaging 7 to 10 mgm, was still recoverable on the 5th puerperal day, but it probably contained little or no pregnanediol glucuronide.

DISCUSSION

The findings of the present study, like those of Venning, Henry and Browne, which they confirm, support the conclusion that the urinary excretion

TABLE II
Clinical data in 5 cases of multiple gestation

Case number	Age	Gravida	Number of fetuses	Maternal health during pregnancy	Menstrual age of pregnancy at termination	Nature of termination
1	22	II	2	Premature separation of placenta at 16 weeks	weeks 17	Hysterotomy
2	20	V	2	Malnutritional anemia and edema	32	Spontaneous labor
3	35	XVI	3	Malnutritional anemia and edema	32	Induced labor
4	33	III	2	Malnutritional anemia and edema	38	Spontaneous labor
5	36	V	2	Malnutritional anemia and edema	39	Spontaneous labor

of pregnanediol during normal pregnancy follows a characteristic course. The daily level of excretion at any stated maturity of pregnancy in a normal patient does not appear to differ markedly from the mean daily norm of that period for a group of patients of comparable body weight. This appears to be true regardless of the size or number of ova *in utero*, though further data from multiple pregnancies are desirable, since our determinations in this type of gestation are the only ones thus far recorded. It must be noted that a majority of women of the "clinic" type who bear more than one fetus at a time suffer from mild malnutritional anemia and hypoproteinemic edema during the late months of pregnancy. All but one of our cases of multiple gestation exhibited this syndrome. Until the effects of such a complication upon the rate of pregnanediol excretion are investigated, the use for physiologic purposes of the results we have obtained must be tentative. So far as our data suffice, they support the suggestion that the maturity of gestation is a more important factor in determining the levels and rates of pregnanediol excretion than is the mass or number of the fetuses and placentas *in utero*.

Our data show that the mean levels of excretion at term are no higher than those observed between the 30th and 32nd week. Throughout the last 8 to 10 weeks the minimal values tend to be roughly constant, and are rarely lower than 60 mgm of pregnanediol per day in normal cases. Continued excretion of less than this amount during the last 10 weeks is therefore definitely abnormal. Diminished excretions of this character have been observed in the "eclampsism" of late pregnancy (6, 8, 10).

The occurrence of the pre-labor drop in output of pregnanediol in all but one of our patients' individual records suggests that it is a characteristic feature of late pregnancy similar to the pre-labor changes in blood volume, body weight and in the concentration of certain metabolites of the blood stream. A similar prepartal fall has been established for the urinary excretion of estrogens (17, 18). In the case of the estrogens Marrian has furthermore noted that the fall is accompanied by a simultaneous drop in the ratio of conjugated estrogen. Although the latter may

not be a constant phenomenon,⁴ its finding raises the question whether the pre-labor fall in output of pregnanediol glucuronide mirrors a true fall in pregnanediol excretion or merely a drop in the proportion of the conjugated steroid. The answer to this must await the further development of methods for the determination of the free steroid in urine.

If it be granted that the output of the glucuronide of pregnanediol reflects the total excretion of this steroid, comparison of the curve of its excretion with that of the estrogens (18) suggests that the pre-labor peak is reached and passed before the occurrence of the similar peak in the curve of estrogen excretion. The final prepartal fall is therefore probably under way at a time when estrogen clearances are still rising to their maximal gestational levels. In so far as these shifts reflect similar shifts in the internal secretion of progesterone and the estrogens, they afford corroborative evidence of changes which have already been postulated for this period of pregnancy because of the nature of the biologic activity of these hormones, and of the particular rôle which each is believed to play in the initiation of labor (19). In this connection it is of interest that Murphy has regularly obtained among parturient women at this pre-labor period evidence of a gradual increase in uterine irritability and in the rate and strength of spontaneous uterine contractions (20).

Browne, Henry and Venning have noted the transient fluctuations which punctuate the rising gestational curve of pregnanediol excretion (3), but have not ventured to read a definite pattern or physiologic significance into such fluctuations. Our data clearly demonstrate that these fluctuations consist of cyclic monthly remissions, the last of which represents the pre-labor fall previously described. Browne and Venning (21) have reported that a similar cyclic pattern characterizes estrogen excretion in pregnancy. Smith and Smith (11), moreover, have stated that such a pattern may also obtain for the gestational curve of pregnanediol excretion, though no experimental data have so far been submitted.

⁴ Personal communication of Dr. J. S. L. Browne.

SUMMARY AND CONCLUSIONS

The urinary excretion of sodium pregnandiol glucuronide was studied by the method of Venning in 6 cases of normal pregnancy at weekly intervals from the 3rd month to full term, and thereafter at daily intervals for the first 5 days of the puerperium. The results confirm the general findings of Venning, Henry and Browne concerning the normal gestational ranges and levels of the excretion of this compound but show, in addition, that the curve of its excretion is characterized by cyclic monthly fluctuations and by a peak in output during the last month. At the time of labor the rate of excretion is falling.

Additional data from 5 cases of multiple pregnancy indicate that the ranges and levels of excretion in these cases are not markedly different from those of single fetus gestation, at least during the last half of gestation.

The findings of this study suggest that the rate of excretion of pregnandiol glucuronide during normal pregnancy is determined by the maturity of gestation rather than by the size or number of fetuses or placentas *in utero*.

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THE EFFECT OF LARGE DOSES OF VITAMINS A, B, C AND D ON THE INCIDENCE OF UPPER RESPIRATORY INFECTIONS IN A GROUP OF RHEUMATIC CHILDREN¹

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Most students of rheumatic fever agree that upper respiratory infections, especially those associated with Group A beta hemolytic streptococci, are often followed by a recurrence of rheumatic symptoms. Any measures which might tend to lessen the incidence of upper respiratory infections among rheumatic subjects are therefore worthy of study.

The occurrence of severe spontaneous infections in animals on vitamin A deficient diets suggested that this vitamin might play an important role in the resistance of individuals to upper respiratory infections (1). Since the characteristic lesion of vitamin A deficiency is the degeneration of specialized epithelium it was thought that the mucous membranes might be peculiarly susceptible in individuals whose diet was low in vitamin A. In spite of numerous studies (2), however, the value of an excess of vitamin A as a means of increasing resistance to upper respiratory infections has not been established, and several observers conclude that there is no justification for calling vitamin A the "anti infective" vitamin (3).

It seemed possible that the addition of several vitamins might be more effective in increasing the general resistance to infection than the addition of vitamin A alone. As far as we have been able to determine from a review of the literature, Stone (4) and her co authors are the only workers who have tried the addition of several vitamins. They studied the effect of adding concentrates of vitamins A, B₁, B₂ and D to the diet of 11 college students for 3½ months, from November 15th until March 1st. The exact dosage of each vitamin was not stated. The students were selected because their general physical condition was poor and they were known to be very susceptible to colds. These authors were of the opinion that

incidence of upper respiratory infections was reduced during the period that the vitamins were given. It is obvious however, that the number of individuals studied was too small to warrant drawing definite conclusions.

Plan of study

It was thought that the effect of the addition of an excess of vitamins to an average diet could be more accurately determined in an institution where the children were under constant medical supervision and where the incidence and types of upper respiratory infections were carefully studied.

PROCEDURE

One hundred and eight rheumatic children, 66 girls and 42 boys ranging in age from 7 to 14 years were admitted during the summer and fall months and remained in the institution from December 1st until the end of May. Children with definite histories of one or two attacks of polyarthritides or carditis without severe cardiac damage were selected. Temperatures and pulse rates were taken three times daily. Leukocyte counts hemoglobin determinations and sedimentation rates were done routinely every 3 to 4 weeks and more often when necessary. Throat cultures to determine the presence of Group A beta hemolytic streptococci were taken every week. Additional cultures were taken on two successive days if a child developed symptoms of any kind. Antistreptolysin titers were also determined at frequent intervals.

The effect of the addition of vitamins A, B complex, C and D was studied during two successive winters.

The 108 children were divided into two groups, one group receiving the regular diet with the addition of vitamins and the other group receiving the regular diet without additional vitamins. The children in the two groups were matched as nearly as possible in regard to rheumatic history age, and general condition.

During 1939 vitamins A, B complex, C and D* were

* During 1939 vitamins A, D and B complex were donated by Lederle Laboratories, Inc. One-half of the vitamin C was given by Chas. Pfizer & Sons (tableted by Lederle Laboratories, Inc.) and remainder by Merck & Co., Inc. During 1940 the vitamins were obtained at cost through the courtesy of Lederle Laboratories, Inc.

¹ This work was aided by a grant from The Commonwealth Fund.

given daily for 5 months from January 1st until June 1st. During the winter of 1939 to 1940, the same vitamins were given daily from December 1st, 1939 until May 1st, 1940. The following dosage was used

Vitamin A	15,000 USP units	} 1 capsule daily, equivalent to 24 cc. cod liver oil	} 8 cc. daily
Vitamin D	1,870 USP units		
Vitamin C	2,000 International units		
Vitamin B ₁	1,000 International units		
B ₂	480 Bourquin-Sherman units		
B ₆	40 "Rat Day" units		
Filtrate (Factor (s))	—approximately 40 Growth units (Rat or Chick)		
Nicotinic Acid or derivatives (pellagra curative)	equivalent to 50 grams of whole liver		

RESULTS

Winter of 1938 to 1939

Beginning in February 1939 and continuing through May, 32 children developed upper respiratory infections associated with the presence of Group A beta hemolytic streptococcus of a single type, Type 4. Most of the children complained of sore throat and had temperatures of 101° to 103° F for 2 or 3 days accompanied by elevated leukocyte counts. Type 4 streptococci were isolated from throat cultures and were usually present in large numbers. The monthly incidence of these infections and their distribution among children on vitamins and among those serving as controls is presented in Table I.

TABLE I
Streptococcus pharyngitis

Months	Total number of cases	Number of children on vitamins since January 1, 1939	Controls
February	4	3	1
March	17	9	8
April	6	5	1
May	5	0	5
	32	17	15

None of the 32 children developed rheumatic manifestations following these upper respiratory infections.

Weight The average gain in weight during the 5-month period for the boys on vitamins and the boys serving as controls was the same. The average gain in weight among the girls receiving vitamins was 15 pounds more than among the girls serving as controls.

Winter of 1939 to 1940

During this year two types of upper respiratory infections were prevalent among the children: an outbreak of influenza³ characterized by fever, malaise and a low leukocyte count, and a series of sore throats accompanied by fever and elevated leukocyte counts. Throat cultures from the influenza cases were uniformly negative for beta hemolytic streptococci. Throat cultures from the cases of pharyngitis in every instance showed Group A beta hemolytic streptococci of a single type, Type 27.

The monthly incidence of influenza and its distribution among children receiving vitamins and those serving as controls is presented in Table II.

TABLE II
Influenza

Months	Total number of cases	Number of children on vitamins since December 1 1939	Controls
February	26	15	11
March	25	11	14
	51	26	25

The monthly incidence from January to May of the cases of streptococcus Type 27 pharyngitis and their distribution among children on vitamins and those serving as controls is presented in Table III.

TABLE III
Streptococcus pharyngitis

Months	Total number of cases	Number of children on vitamins since December 1 1939	Controls
January	15	6	9
February	2	1	1
March	6	4	2
April	1	0	1
	24	11	13

³ Dr. Thomas Francis of New York University College of Medicine was kind enough to examine material from several of these cases for the presence of influenza virus. A strain of epidemic influenza virus was isolated. Neutralization tests with acute and convalescent sera from 3 patients showed a rise in antibody titer during convalescence.

Comparison of rheumatic activity in children receiving vitamins and those serving as controls

Of the 13 children (Table III) who were not receiving vitamins and who contracted Type 27 infections, only one developed a rheumatic recurrence following a latent period

Of the 11 children who were receiving vitamins and who contracted Type 27 infections, 3 developed rheumatic recurrences following a latent period. One of these 3 children had the Type 27 pharyngitis on January 17 1940 when he had been receiving vitamins for 53 days. He developed rheumatic symptoms on February 9, 1940 after a latent period of 18 days. The other 2 children developed pharyngitis associated with streptococcus Type 27 on March 25 and March 26 1940 respectively when they had been receiving vitamins for nearly 4 months. The pharyngitis in these 2 girls was followed by rheumatic manifestations in 1 the latent period was 22 days, and in the other 20 days. The administration of vitamins in these 3 children was not interrupted at any time.

In view of the work of Rinehart and his co-workers and of others (5) it is of interest that these 3 children developed rheumatic recurrences in spite of the administration of large amounts of vitamin C for a considerable period of time before the rheumatic manifestations. All 3 of these children had been here during two successive winters (1938 to 1939 and 1939 to 1940) and had received vitamins for a 5-month period during both years. Since the number of children who were on vitamins and who developed rheumatic symptoms was so small and since no chemical studies were made to determine how well the cevitamic acid was retained by these children, no conclusions in regard to the value of vitamin C in preventing the development of rheumatic fever can be drawn. However, our findings, limited as they are, are in accord with those of other workers (6).

Weight In contrast to the previous year, the average gain in weight of the boys receiving vitamins was slightly greater than of the boys serving as controls. On the other hand the gain in weight among the two groups of girls was the same. Children receiving institutional care usually tend

to gain and no definite improvement attributable to the additional vitamins was apparent.

DISCUSSION

In previous studies to determine the value of one or more vitamins in reducing the incidence of upper respiratory infections the subjects in most instances have been ambulatory, and it was therefore, difficult to be certain that the vitamins had actually been taken. In some of the reports the individuals who received the vitamins were selected because they were thought to be unusually susceptible to colds. In cases where a reduction in the incidence of upper respiratory infections seemed related to the intake of increased amounts of vitamins it was usually based on the history given by the individual as to the number of colds he had had during the previous winter. In some of the reports no studies of control groups were included.

In this study a group of 108 rheumatic children ranging in age from 7 to 14 years was studied under constant conditions from December 1st until the end of May. The children lived and went to school in the same building. They had no contact with other children, and each child was only permitted two adult visitors every 6 weeks.

The administration of the vitamins was carefully supervised by the nursing staff. The children in the control group and in the group receiving vitamins were matched as closely as possible in regard to age, rheumatic history and general condition so as to be comparable. Twenty-one children 9 boys and 12 girls, who had received vitamins during 1939 and who remained here during 1940, were given vitamins two years in succession.

During each of the two winters of the study cases of pharyngitis associated with a single type of streptococcus (Type 4 in 1939 and Type 27 in 1940) were prevalent. The incidence of these infections in each of the years among the children receiving vitamins and those in the control group was essentially the same.

During February and March 1940, 51 children had influenza, 26 of these children were receiving vitamins and 25 were in the control group.

There was no significant difference in weight gain between the two groups.

CONCLUSIONS

1 No evidence was obtained to suggest that the addition of large doses of vitamins A, B complex, C and D to an ordinary well-balanced diet reduces the incidence of upper respiratory infections

2 Three children who had received the additional vitamins for a considerable period of time developed rheumatic symptoms following an attack of streptococcus pharyngitis

3 Children on the regular diet without additional vitamins and those on the regular diet with additional vitamins gained weight at approximately the same rate during the 5-month period

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CARBOHYDRATE METABOLISM IN ADDISON'S DISEASE¹

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Our understanding of the nature of the disordered carbohydrate metabolism in Addison's disease has advanced little since the original demonstration by Porges in 1910 (1) of the frequent occurrence of hypoglycemic reactions and the studies of Eppinger, Falta and Rudinger in which an increase in glucose tolerance was noted (2). Subsequently, however it was shown that patients with Addison's disease developed marked symptoms of hypoglycemia following the administration of small doses of insulin (3) and failed to show a rise of blood sugar as great as normal subjects following the injection of a standard dose of epinephrin (4).

Recently Long Katzin and Fry (5) reported in detail their studies relating to the effect of adrenal cortical hormone on the carbohydrate metabolism of experimental animals. From their own studies, as well as those of others these authors concluded that "the administration of cortical extract apparently decreases the proportion of glucose oxidized while there is an increased proportion deposited as liver glycogen.

It is suggested that one of the properties of the cortical hormone is a stimulation of protein catabolism and that the increased carbohydrate levels nitrogen and potassium excretion following its injection into animals is an expression of this effect.

A study of carbohydrate metabolism in untreated patients with Addison's disease is difficult because of the poor clinical condition of these patients. In the present study we have had the good fortune to be able to complete carefully controlled metabolic studies on a limited number of untreated patients and on a larger number of patients who were maintained in good condition by

means of synthetic desoxycorticosterone acetate therapy.³

METHODS

Before these studies were undertaken, normal subjects and patients with Addison's disease were maintained for a period of 1 to 2 weeks on a standard diet of adequate caloric intake. The ratio of carbohydrate protein fat was kept constant. In most instances patients received 50 grams of carbohydrate, 1.3 grams of protein and 1.5 grams of fat per kgm. of body weight.

Respiratory metabolism

During a preliminary period of 1 to 2 weeks both the normal subjects and patients were trained for respiratory metabolic studies. Expired air was collected for 10-minute periods in a modified Baily gasometer and samples were analyzed in duplicate for carbon dioxide and oxygen by means of a Haldane Henderson gas analyzer. Differential derivation of calories was made with the aid of Lusk's table (6). In addition to determining the standard metabolism after a 15-hour fast, the respiratory metabolism blood sugar (7) and urinary nitrogen (Kjeldahl) were determined throughout the day at 2 hour intervals during a total fasting period of 22 hours. These determinations were repeated following the injection of adrenal cortical extract (Wilson, 50 cc.)⁴ The respiratory quotient, blood sugar and urinary nitrogen excretion were determined at hourly intervals following the intravenous administration of glucose before and after treatment with adrenal cortical extract (50 cc.) desoxycorticosterone acetate (30 mgm.) corticosterone (85 mgm.)⁵ and 17 hydroxy 11-dehydrocorticosterone (33 mgm.) (Kendall's Compound E)⁶

Standard intravenous glucose tolerance test

A test was adopted in which 0.5 gram of glucose per kgm. of body weight was injected intravenously as a 20

¹ The synthetic desoxycorticosterone acetate used in this study was provided through the courtesy of Doctor E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

² The adrenal cortical extract used in this study was supplied by Doctor David Klein of the Wilson Company Chicago Ill.

³ We are indebted to Doctor E. C. Kendall of the Mayo Clinic, Rochester Minn., for the crystalline corticosterone and 17 hydroxy 11-dehydrocorticosterone (Compound E)

¹ This study was aided by a grant from the Committee on Research in Endocrinology National Research Council.

² John D. Archbold, Fellow in Medicine.

per cent solution in distilled water. The flow was adjusted so that the infusion was completed in 30 minutes. This rate of glucose administration (0.5 gram per kgm. per $\frac{1}{2}$ hour) approximated the maximum rate of intestinal absorption of glucose and hence provided an intravenous glucose test which was within physiological limits. Capillary blood for sugar determinations was taken in the fasting state and at 30-minute intervals during a 4-hour period following the glucose infusion. Urine specimens were collected at appropriate intervals and analyzed for sugar.

Oral glucose tolerance test

A solution of glucose, 175 grams per kgm of body weight, was given. Capillary blood for sugar determinations was taken during the fasting state and 30, 60, 120 and 180 minutes after the ingestion of glucose.

Epinephrin test

Epinephrin, 0.007 mgm. per kgm of body weight, was injected subcutaneously. The site of the injection was massaged vigorously for a period of 2 minutes. Capillary blood for sugar determinations was taken during the fasting state and 15, 30, 60 and 90 minutes after the injection of epinephrin.

OBSERVATIONS

Increased appetite for carbohydrate

It is of interest to note that many patients with Addison's disease had formed the habit of supplementing their regular diet with intermediate nourishment of foods containing readily available carbohydrate (Patient E L, see protocol). In this respect there is a parallel to the increased appetite for salt and salty foods which has been noted clinically by many observers, and confirmed experimentally by the studies of Richter and Eckert on adrenalectomized rats (8).

Fasting blood glucose

The fasting blood glucose level of untreated patients with Addison's disease was observed to be in the low-normal range in most instances. In a group of 20 patients the average blood glucose value* (108 determinations) was observed to be 80 mgm per 100 cc before treatment. Prolonged treatment with desoxycorticosterone acetate did not affect the fasting blood glucose level since the average value observed during treatment was also

* All blood sugar values determined by the modified micromethod of Folin and Malmros are arbitrarily referred to in this study as "blood glucose values"

80 mgm per 100 cc (166 determinations). These observations confirm earlier studies (9, 10).

Treatment with adrenal cortical extract (Wilson, 4 to 10 cc daily, injected subcutaneously) was associated with an appreciable rise in the average fasting blood glucose level (Table I).

TABLE I
Effect of adrenal cortical hormone therapy on
fasting blood glucose
(Addison's disease)

	Fasting blood glucose (mgm. per 100 cc.)					
	Before treatment	Desoxy corticosterone acetate*	Adrenal cortical extract (injected)	Adrenal cortical extract (oral)	Corticosterone (injected)	17 hydroxy-11-dehydrocorticosterone (injected)
		3-20 mgm. daily	4-10 cc. daily	8-12 cc. daily	85 mgm.	33 mgm.
5 patients (Number of determinations)	76 (43)	77 (60)	81 (20)	83 (30)		
Patient E L.	75	75	84 99†		99	95
Range	(68-81)	(72-81)				

* Includes values observed during treatment with either daily intramuscular injection of a solution of hormone in oil, or subcutaneously implanted pellets of crystalline desoxycorticosterone acetate.

† Massive dose, 50-70 cc, during a 24 hour period.

The oral administration of larger quantities of adrenal cortical extract in glycerol solution (8 to 12 cc daily, 1 cc representing 50 grams of adrenal gland) was likewise associated with a rise in the average value for fasting blood glucose. Treatment with large quantities of adrenal cortical extract (Wilson, 20 to 50 cc daily, injected subcutaneously and intravenously), corticosterone (85 mgm) and 17-hydroxy-11-dehydrocorticosterone (33 mgm) in oil, injected intramuscularly in divided doses, was followed by a much greater elevation in fasting blood glucose level (Patient E L, Table I).

The efficacy of treatment with adrenal cortical extract (50 to 70 cc) in preventing the gradual fall in blood glucose during a prolonged fast is illustrated in Figure 1. The rise in blood glucose which reached its maximum 6 to 8 hours after injection of extract did not appear to be due to traces of epinephrin which may have been present in the extract, since there was no immediate rise in blood glucose as might have been anticipated following an injection of epinephrin.

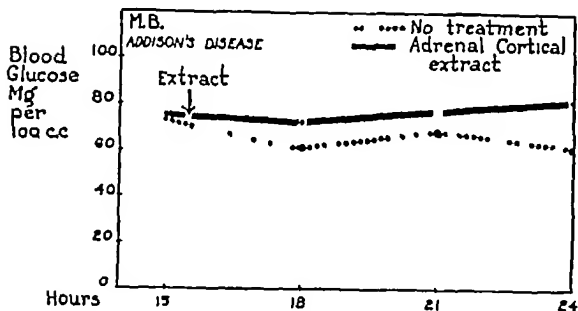


FIG. 1. EFFECT OF ADRENAL CORTICAL EXTRACT ON BLOOD GLUCOSE LEVEL (PROLONGED FAST)

Oral glucose tolerance

The striking intolerance of patients with Addison's disease to the oral administration of glucose is well known (11). Not infrequently an adrenal crisis has been precipitated by this test.

A comparison between the blood glucose values of 8 control subjects and 4 untreated patients with Addison's disease following the oral administration of glucose, is shown in Figure 2. The low-normal initial fasting sugar level, the "flat type" of glucose curve, and the striking hypoglycemia which was frequently noted 2 to 3 hours following the administration of glucose are of particular

significance in the response of patients with Addison's disease. At the time of initial examination approximately 75 per cent of a group of 30 patients with Addison's disease were found to have an abnormal oral glucose tolerance curve.

The symptoms of pylorospasm which so frequently followed the oral administration of glucose to these patients suggest that the apparent increase in glucose tolerance (flat type of curve) was in a large measure due to an abnormality in the intestinal absorption of glucose. This was confirmed by the normal height to which the blood glucose level rose following the intravenous ad-

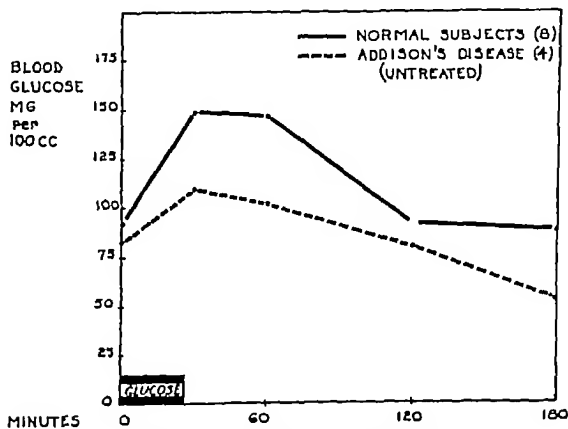


FIG. 2. ORAL GLUCOSE TOLERANCE TEST

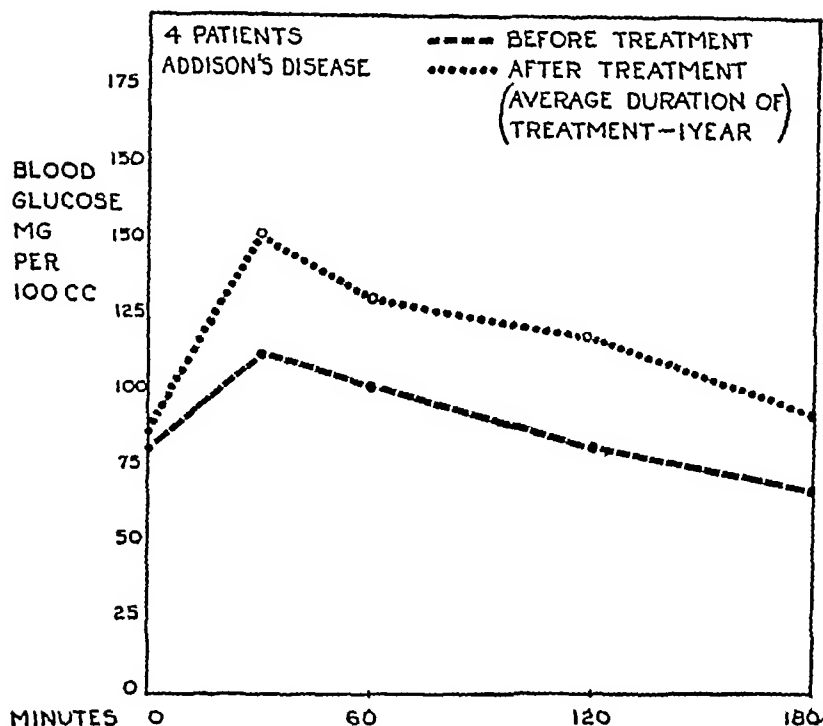


FIG 3 ORAL GLUCOSE TOLERANCE TEST THE EFFECT OF
DESOXYCORTICOSTERONE TREATMENT

ministration of a standard dose of glucose (see Figure 5)

Following prolonged treatment (1 year or more) with desoxycorticosterone acetate, we observed a marked change in the oral glucose tolerance curve in 4 patients (Figure 3). The clinical condition of these patients was markedly

improved at the time the second glucose curve was determined. It would seem likely that the change in the glucose curve was due primarily to improved absorption of glucose from the intestinal tract and not to a marked or specific effect of desoxycorticosterone acetate on the internal metabolism of glucose, inasmuch as desoxycortico-

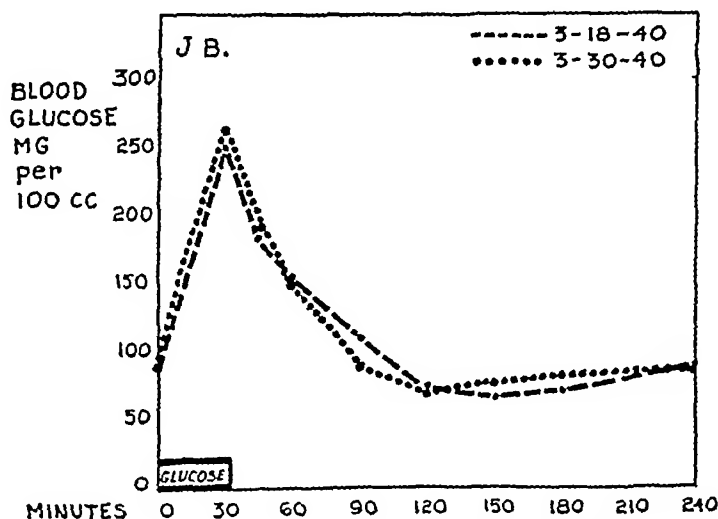


FIG 4 INTRAVENOUS GLUCOSE TOLERANCE TEST
NORMAL SUBJECT

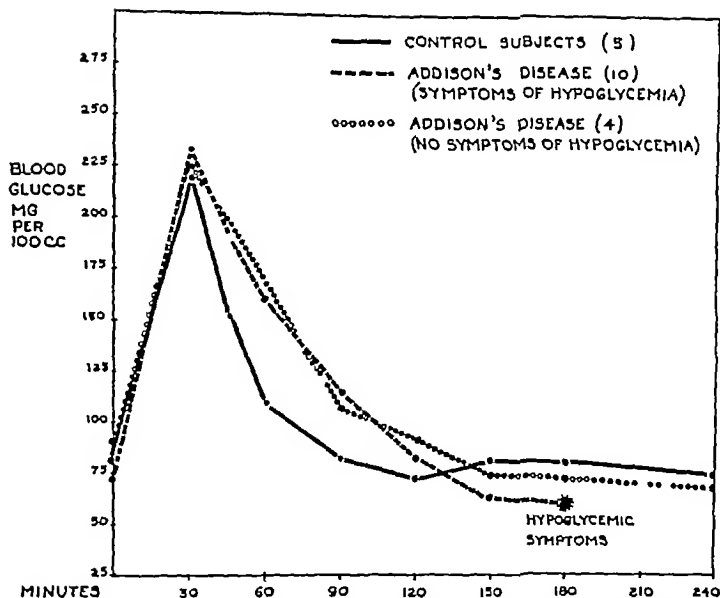


FIG. 5 INTRAVENOUS GLUCOSE TOLERANCE TEST IN PATIENTS WITH ADDISON'S DISEASE

sterone acetate therapy had no demonstrable effect on the blood glucose curve following the intravenous administration of glucose.

Intravenous glucose tolerance

In order to eliminate the obvious disadvantages of glucose administered orally, we used a standard intravenous glucose tolerance test (see methods). One consideration seemed essential, *viz*, that the rate of glucose solution introduced intravenously should not exceed the maximum rate of intestinal absorption of glucose, if physiological studies were to be made. Experience now indicates that, under constant conditions, the blood glucose curve following the intravenous administration of 0.5 gram of glucose per kgm. of body weight during a 30-minute period is constant for a given individual (Figure 4).

Patients with Addison's disease frequently developed marked symptoms of hypoglycemia 2 to 3 hours after the administration of glucose intravenously (Table II). In some patients coma and vasomotor collapse occurred and additional glu-

TABLE II

Intravenous glucose tolerance curve

PATIENTS WITH ADDISON'S DISEASE															
Pa- tient	Sex	Age	Blood glucose (mgm. per 100 cc.)												
			Fast- ing	30 min utes	45 min- utes	60 min- utes	90 min- utes	120 min- utes	150 min- utes	180 min- utes	240 min- utes				
F. H.	M	21	77	210	182	163	123	86	65	50	48*				
V. E.	F	39	79	225	177	170	118	84	66	59	59*				
S. W.	F	55	111	426	235	171	100	77	60*						
J. P.	M	21	79	270	202	192	163	99	75	62*					
M. B.	F	31	78	250	135	77	56	48*	61*						
O. O.	M	43	78	241	182	140	100	74	52	50*					
R. L.	F	31	76	233	183	153	103	77	61	50*					
A. H.	M	39	89	241	182	149	118	90	76	68	62				
O. G.	M	62	81	264	215	196	142	105	86	69	69				
B. W.	M	26	78	200	164	131	105	84	68	62	77				
S. P.	M	27	80	206	156	105	80	68	60	72					
A. H.	F	20	78	222	194	164	131	97	70	60	64				
H. H.	M	38	99	254	225	153	112	104	78	78	80				
NORMAL SUBJECTS															
F. A.	M	23	81	282	204	164	88	67	69	82	106				
A. K.	M	23	84	194	123	91	78	71	76	84					
C. D.	M	26	89	220	123	70	55	78	80	84	87				
R. L.	M	28	95	290	202	164	106	75	73	64	68				
S. A.	M	25	87	220	153	110	81	76	83	81	78				

* Hypoglycemic reaction.

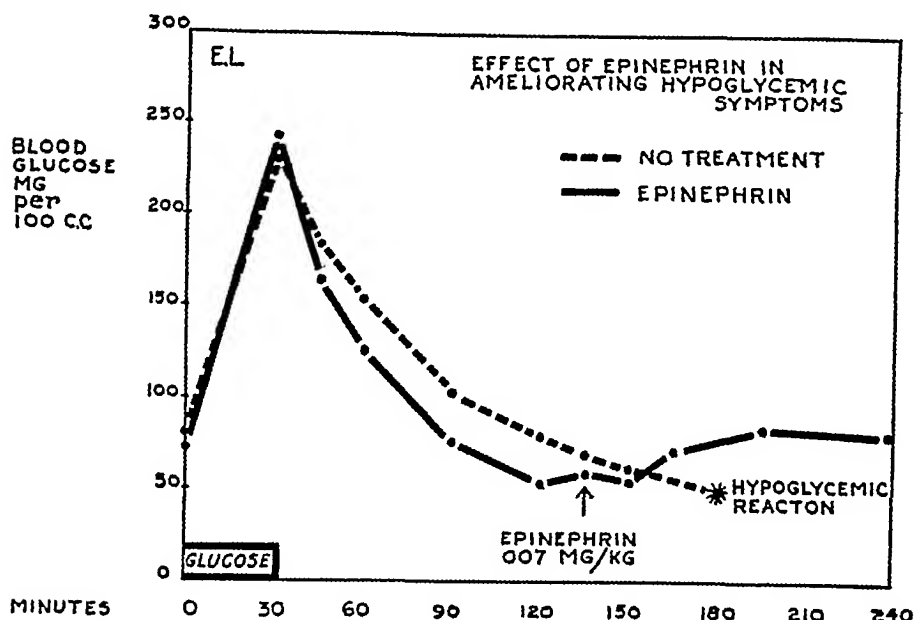


FIG 8 INTRAVENOUS GLUCOSE TOLERANCE TEST (ADDISON'S DISEASE)

and more prolonged action of adrenal cortical hormone (4 to 24 hours)

In one patient the response to epinephrin following an overnight fast was observed in the untreated state and again after several days of treatment with adequate doses of adrenal cortical hormone (20 cc daily, injected subcutaneously). Associated with treatment there was a small but definite increase in the height of the blood glucose curve, an increment of 27, 15, 11, 3 mgm, respectively, at 15, 30, 60 and 90 minutes following the subcutaneous injection of epinephrin (see methods)

Response to insulin

Because of the profound reaction of patients with Addison's disease to relatively small doses of insulin, we did not study the action of this hormone in human subjects. In bilaterally adrenalectomized dogs (to be reported elsewhere), it was demonstrated that treatment (6 to 18 hours beforehand) with 50 to 80 cc of adrenal cortical extract completely prevented the severe and often fatal hypoglycemic reaction which followed the intravenous injection of 0.25 units of insulin per kgm. Large quantities of desoxycorticosterone acetate (50 mgm or more) were ineffective in preventing convulsions. Treatment with 17-hydroxy-11-dehydrocorticosterone, however, in a dose of 10 mgm, permitted the animal to survive

the test without supplementary glucose therapy. The limited quantity of crystalline hormone available did not permit an exact determination of equivalent quantities of adrenal cortical extract and crystalline 17-hydroxy-11-dehydrocorticosterone

Standard metabolism

The standard metabolism of patients with Addison's disease has been considered to be definitely lower than that of normal subjects. The values which were obtained in 15 untreated patients are given in Figure 9. In our experience a basal

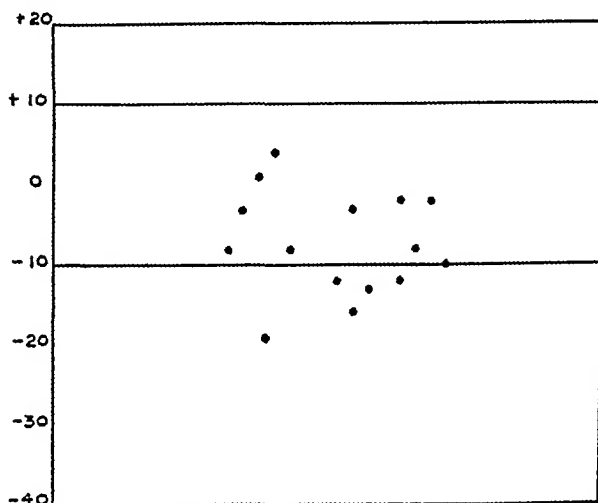


FIG 9 BASAL METABOLIC RATE IN 15 PATIENTS WITH ADDISON'S DISEASE (UNTREATED)

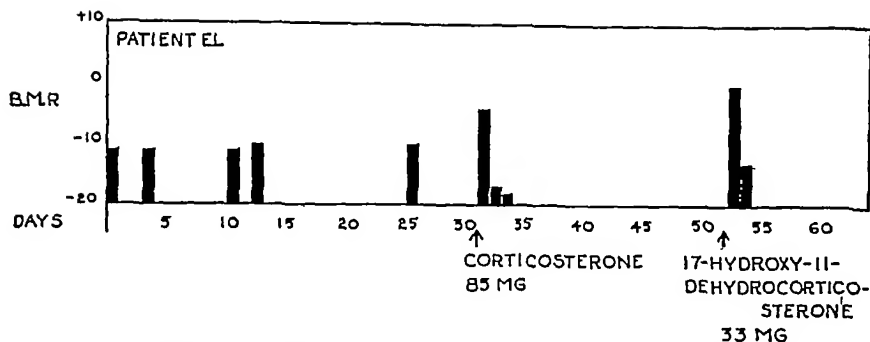


FIG. 10. THE EFFECT OF ADRENAL CORTICAL HORMONE TREATMENT ON BASAL METABOLIC RATE (ADDISON'S DISEASE)

metabolic rate lower than -20 per cent is unusual in patients with Addison's disease uncomplicated by thyroid or pituitary deficiency

In a group of 7 patients, no significant change in the average basal metabolic rate was noted following continued treatment with desoxycorticosterone acetate. The average value before treatment was -10 per cent (18 determinations) and during treatment -12 per cent (10 determinations). However, treatment with corticosterone or 17 hydroxy-11-dehydrocorticosterone was followed by a significant rise in the standard metabolism of Patient E. L. (Figure 10)

Standard respiratory quotient

A comparison between the standard respiratory quotient of 6 normal subjects and 8 patients with Addison's disease revealed no significant difference between the average of the two groups (Table III). However, if the values for 3 patients (S. P., A. H. and A. S.) who were observed to have no demonstrable abnormality in carbohydrate metabolism (see intravenous glucose tolerance tests, Table II) are excluded the average value for the group of patients with the "carbohydrate-defect" is approximately 0.90. This value is significantly higher than the value for the group of control subjects.

Prolonged treatment with desoxycorticosterone acetate (maintenance dose) had no effect on the standard respiratory quotient of 3 patients (E. L., A. H., M. B., Table IV). Treatment with large

TABLE III
Standard respiratory quotient
(Constant dietary regimen)

ADDISON'S DISEASE									
Patient	Sex	Age	Weight	Respiratory quotient					
			kgm.						
E.L	F	51	48	0.92	0.90	0.93	0.91	0.85	0.87
				0.90	0.92				
J.P	M	21	45	0.90	0.90				
M.B	F	31	48	0.88	0.86	0.86	0.87		
Y.E	F	39	54	0.90					
O.O	M	43	59	0.92					
A.S	M	33	57	0.87	0.92	0.87	0.86		
S.P	M	27	70	0.86	0.83	0.79			
A.H	F	20	57	0.85	0.86	0.83	0.84		
Weighted average				0.88					
NORMAL SUBJECTS									
F.A.	M	21	60	0.89					
S.A.	M	23	81	0.84					
R.L	M	28	75	0.82	0.82	0.80			
C.D	M	26	84	0.85	0.86				
V.H	F	30	47	0.87					
J.B	F	37	58	0.88	0.86				
Weighted average				0.86					

quantities of adrenal cortical extract (20 to 70 cc.) corticosterone (85 mgm) or 17 hydroxy-11-dehydrocorticosterone (33 mgm) was followed by a striking reduction in the standard respiratory quotient when treatment was instituted 12 to 18 hours preceding the test (Patient E. L. Table IV). The reduction in standard respiratory quotient was accompanied by a rise in fasting blood

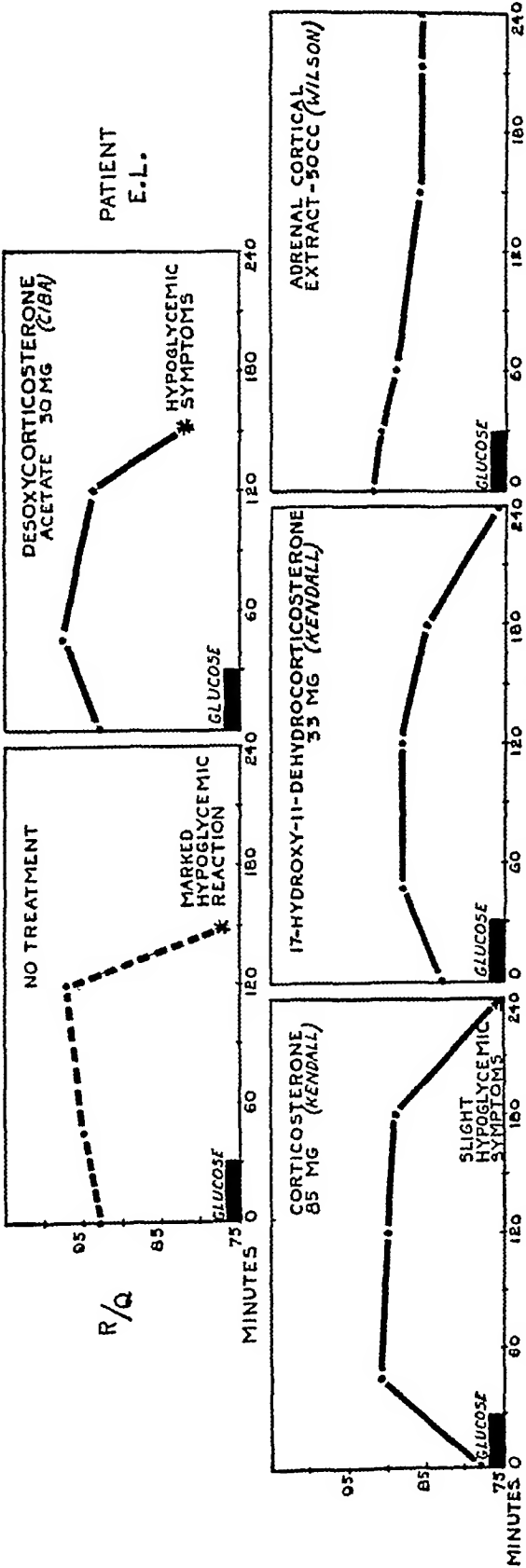


FIG 13 THE EFFECT OF ADRENAL CORTICAL HORMONE ON R/Q FOLLOWING INTRAVENOUS GLUCOSE (ADDISON'S DISEASE)

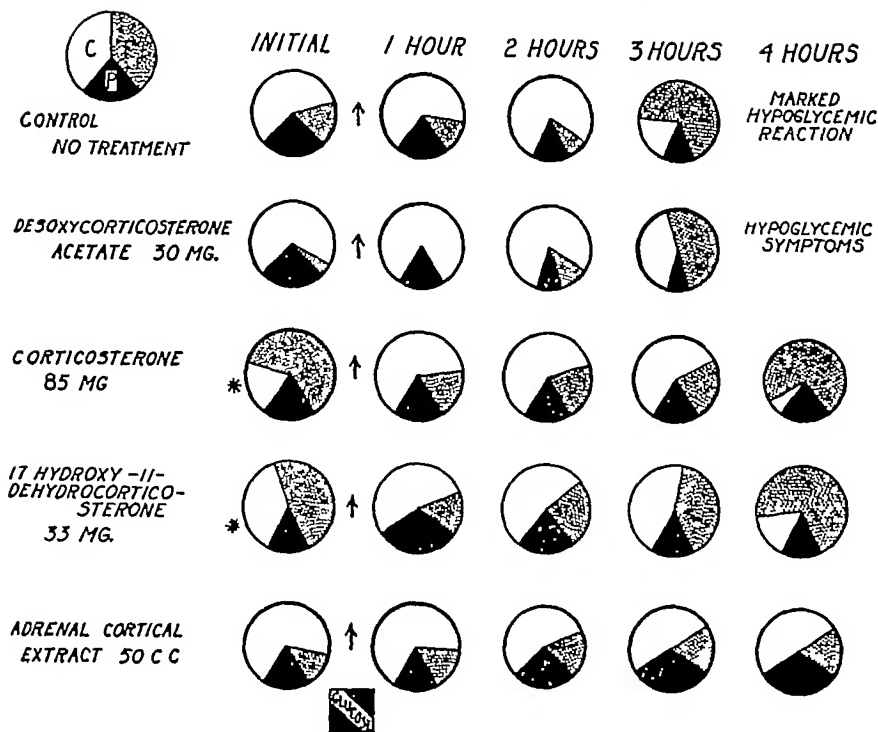


FIG. 14 ADDISON'S DISEASE. E. L. CALORIC CHANGES FOLLOWING INTRAVENOUS GLUCOSE

erately large dose of this compound (30 mgm.) was not associated with any change in the respiratory quotient. Treatment with 50 cc. of adrenal cortical extract, injected 4 to 6 hours previous to the test, did not affect the initial standard respiratory quotient but was associated with a marked change in the subsequent respiratory quotient values (Figure 13, Patient E. L.) In another experiment (Figure 11) it was observed that treatment with 50 to 70 cc. of adrenal cortical extract during a 12- to 24-hour period preceding the test significantly affected the initial respiratory quotient. Treatment with corticosterone and with 17 hydroxy 11-dehydrocorticosterone (dissolved in oil and injected intramuscularly during a 12-hour period prior to the standard intravenous glucose tolerance test) was followed by a marked reduction in initial standard respiratory

quotient, as well as in the values following the intravenous glucose tolerance test (Figure 13)

Differential derivation of calories (Lusk's table) indicated that in the untreated state (Patient E. L. Figure 14) a high proportion of calories was derived from carbohydrate oxidation following the intravenous administration of glucose, particularly during the second hour of the test. During the third hour of the test, in the untreated patient, there was a sudden reduction in the proportion of calories derived from carbohydrate (low R/Q) immediately preceding the onset of marked symptoms of hypoglycemia. Treatment with desoxycorticosterone acetate had no effect on these changes. Treatment with adrenal cortical extract, 17 hydroxy-11-dehydrocorticosterone and corticosterone (in order of potency) was followed by a significant reduction in the proportion of

weakness and nervousness progressing rapidly to marked mental confusion. The following data obtained at this time indicated that the reaction was specifically related to a disturbance in carbohydrate metabolism and not to a loss of sodium, chloride and water with subsequent dehydration.

1 No appreciable loss in body weight during the 3 days of the experiment

2 Rapid recovery following intravenous glucose therapy without additional sodium chloride or adrenal cortical hormone therapy

3 Hypoglycemia (55 mgm) was associated with a normal concentration of sodium and chloride in the blood with evidence of only slight hemoconcentration: serum sodium 140 m eq, chloride 101.4 m eq, and potassium 6.1 m eq, per liter, carbon dioxide combining power 49 volumes per cent, non-protein nitrogen 27 mgm per 100 cc. The urine contained acetone bodies 4 +

The slightly higher-than-normal serum potassium value may be of some significance in association with the low blood glucose level (13).

Thus within a period of 72 hours, during which he received a diet of adequate caloric content, and during a period in which blood pressure, plasma volume and electrolyte balance were maintained by means of desoxycorticosterone acetate treatment, this patient developed a severe hypoglycemic reaction. This study demonstrates the decreased glycogenesis which is associated with Addison's disease, as well as the inability of desoxycorticosterone acetate treatment to increase the formation of glucose from non-carbohydrate sources.

Hypoglycemia precipitated by intercurrent infections

Acute respiratory infections and gastro-enteritis constitute the most frequent complications which precipitate hypoglycemia in patients with Addison's disease. If not recognized and treated this complication may result in a fatal outcome.

In the absence of complications, treatment with desoxycorticosterone acetate maintained a majority of patients with Addison's disease (12) in good clinical condition without supplementary treatment with specific carbohydrate-regulating factor. The frequent ingestion of food provided an ade-

quate supply of readily available carbohydrate. However, when this source of supply was restricted (fasting, nausea, vomiting), or when the demand for carbohydrate was increased (fever, infection), the blood glucose level rapidly declined and severe hypoglycemic symptoms appeared despite adequate desoxycorticosterone acetate therapy. The reasons for the rapid onset of hypoglycemia in these patients appeared to be two-fold: (a) oxidation of carbohydrate which rapidly depleted available stores, and (b) inability to form adequate quantities of carbohydrate from non-glucose sources.

At present the only form of specific hormone therapy possessing carbohydrate-regulating factor which is available commercially is adrenal cortical extract. This must be used in large doses, 20 to 50 cc daily, in the presence of severe infection. In addition, it is desirable to provide carbohydrate by parenteral administration of glucose solutions, if patients are unable to take adequate quantities of fruit juice, lactose, gingerale, etc., by mouth.

Lactic acid

Because of the position of lactic acid as a transition substance between protein and glycogen, and its importance in glucose oxidation, it seemed desirable to study its glycogenic action in patients with Addison's disease and in adrenalectomized animals.

In Patient O O treatment with lactic acid (18 grams of racemic lactic acid administered intravenously as the sodium salt dissolved in 1000 cc of water) failed to alleviate the symptoms of hypoglycemia which were initiated by the standard intravenous glucose tolerance test. Glucose administered intravenously 1 hour after the lactate solution had been started promptly corrected the hypoglycemia. These observations on patients were confirmed by experiments on adrenalectomized dogs (14) in which *D*-lactic acid administered intravenously failed to prevent the convulsions induced by insulin, whereas treatment with glucose completely prevented signs of hypoglycemia. Direct evidence of the diminished ability of untreated adrenalectomized animals to convert lactic acid to glucose has also been obtained in phlorhizinized adrenalectomized rats (14).

*Physiological activity of steroid compounds
obtained from the adrenal cortex*

To date numerous crystalline compounds have been isolated from adrenal cortical extracts. In the present study we have compared the effect of three of these compounds on carbohydrate metabolism in man. Desoxycorticosterone, which appears to be the most active adrenal cortical compound thus far isolated as measured by its ability to maintain the life of adrenalectomized animals, is very potent in electrolyte regulating property (9). It appeared from this study that desoxycorticosterone treatment had little, if any specific effect on carbohydrate metabolism in man. Corticosterone a compound similar to desoxycorticosterone, but with an additional hydroxyl group, probably at C_{11} , had less electrolyte regulating property (15) than desoxycorticosterone, but it was much more potent in its carbohydrate-regulating effect. Seventeen hydroxy 11-dehydrocorticosterone was more potent in carbohydrate regulating property than either corticosterone or desoxycorticosterone, but appeared from animal experiments to be much less effective than either of these compounds in regulating electrolyte balance and in maintaining the life of adrenalectomized animals. Thus oxygenation of desoxycorticosterone in positions 11 and 17 greatly increased carbohydrate regulating potency but at the same time greatly decreased electrolyte regulating and life-maintaining properties.

Potent adrenal cortical extracts appeared to contain appreciable quantities of both electrolyte and carbohydrate regulating factors. However in man relatively large quantities of extract must be given to demonstrate either effect. Doctor E. C. Kendall of the Mayo Clinic kindly supplied data regarding the approximate steroid content of Wilson's adrenal extract (50 cc. contains approximately 3 mgm. corticosterone, 3 mgm. dehydrocorticosterone, 3 mgm. 17 hydroxy 11-dehydrocorticosterone, 1 mgm. 17 hydroxycorticosterone, as well as unidentified substances). Our experiments suggest that the carbohydrate-regulating potency of this extract (50 cc. being as effective as 85 mgm. of corticosterone or 33 mgm. of 17 hydroxy-11-dehydrocorticosterone) is much greater than can be explained by its content of these two constituents.

DISCUSSION

A comparison between the oral and intravenous glucose tolerance curves indicates that the so-called flat-curve following orally administered glucose actually represents delayed or poor absorption of glucose. Similar changes have been observed in a patient with idiopathic steatorrhea (non tropical sprue). It appears from these studies that a 'flat-curve' following orally administered glucose is of little diagnostic significance.

The striking hypoglycemia which occurs in many patients with Addison's disease following the standard intravenous glucose tolerance test is of great clinical significance since it implies the existence of a marked disturbance in carbohydrate metabolism. The test is of considerable aid in diagnosis, *although it is not specific*. Its greatest usefulness lies in the ease with which it permits a disturbance in carbohydrate metabolism to be detected. The changes which were observed in patients following intravenous glucose administration were in many ways similar to the changes observed by Kendall *et al* (13) in adrenalectomized dogs. A comparison between intravenous glucose tolerance curves performed before and after specific hormone therapy indicates that with adequate treatment there is a marked increase in the threshold at which severe hypoglycemic symptoms occur. Thus the adequately treated patient with Addison's disease and the normal subject frequently do not manifest symptoms at a blood glucose level which may be associated with marked signs and symptoms of hypoglycemia in the untreated patient. The exact mechanism for this difference in threshold is not known at present. Its clinical significance, however, is apparent.

The increase in fasting blood glucose level which was observed following the administration of crystalline corticosterone and 17 hydroxy-11-dehydrocorticosterone precludes the inference that a rise in blood glucose following adrenal cortical hormone therapy is necessarily due to contaminating substances *viz* epinephrine.

The increased height to which the blood glucose level rose and the glycosuria which was observed following specific hormone therapy in Patient E. L. (Figure 6) substantiate the studies of Lukens (16) and others regarding the nature of

the diabetes in patients with adrenal cortical hyperfunction (tumor and hyperplasia)

Preliminary studies on lactic acid metabolism indicate that the ability of patients with Addison's disease to form glucose from this substance is greatly impaired. These studies have been confirmed experimentally in phlorhizinized rats and, in addition, it appears that the formation of glucose from pyruvate and from glycogenic amino acids is also impaired (14). Treatment with potent adrenal cortical hormone restores to normal the capacity of these animals to form glucose from lactic acid, pyruvate and glycogenic amino acids.

It is suggested that in adrenal cortical insufficiency carbohydrate oxidation is adequate, although the ability to form glucose and glycogen from intermediate products of carbohydrate and protein metabolism is impaired. As a result, the glycogen depots in the body are soon exhausted, and ingested preformed carbohydrate is required to maintain the blood glucose level and to provide a readily available source of energy. Hormone therapy increases the ability of the body to form glucose and glycogen from the intermediate products of both carbohydrate and protein metabolism and apparently increases the proportion of calories derived from protein and fat.

SUMMARY

Some or all of the following abnormalities in carbohydrate metabolism were observed in a large proportion of patients with Addison's disease

- 1 A low-normal fasting blood glucose level
- 2 Striking hypoglycemia
 - (a) Following the oral or intravenous administration of glucose
 - (b) Following a 24-hour fast
 - (c) During fever or infections
 - (d) On a diet high in fat and low in carbohydrate
- 3 Decreased threshold at which hypoglycemic symptoms appeared
- 4 Flat type of oral glucose tolerance curve
- 5 Absence of "rebound" in blood glucose curve following the administration of intravenous glucose
- 6 Decreased glycemic response to epinephrin

- 7 High standard respiratory quotient
- 8 Increase over normal in respiratory quotient following glucose administration
- 9 Low-normal basal metabolic rate

With the exception of its effect on the oral glucose tolerance curve, the abnormalities in carbohydrate metabolism were not significantly altered by desoxycorticosterone acetate (Ciba) therapy. Treatment with large quantities of adrenal cortical extract (Wilson, 50 cc), 17-hydroxy-11-dehydrocorticosterone (Compound E, Kendall, 33 mgm), and corticosterone (85 mgm), in order of potency, was observed to

- (a) Increase the fasting blood glucose level
- (b) Decrease the standard respiratory quotient
- (c) Increase the blood glucose level and renal excretion of nitrogen and decrease the respiratory quotient following the standard intravenous glucose tolerance test
- (d) Increase the threshold at which hypoglycemic symptoms appeared
- (e) Increase the basal metabolic rate

Treatment with adrenal cortical extract was also observed to

- (a) Increase the glycemic response to epinephrin
- (b) Increase the fasting blood glucose level during fever and infections

These experiments suggest that the adrenal cortical hormone has a direct action on carbohydrate metabolism in man, and are in agreement with the animal experiments reported by Long, Katzin and Fry (5). Our experimental data suggest that the adrenal cortical hormone increases the ability of the organism to form glucose and glycogen from intermediate products of both carbohydrate and protein metabolism and in this manner regulates the utilization of carbohydrate.

CONCLUSION

A high proportion of untreated patients with Addison's disease was observed to have a disturbed carbohydrate metabolism. This defect could be most readily demonstrated by the application of a standard intravenous glucose tolerance test. The abnormality in carbohydrate metabolism appeared to be specific since it persisted de-

spite the correction of the disturbance in electrolyte balance, plasma volume and blood pressure by means of desoxycorticosterone acetate therapy. Treatment with large quantities of adrenal cortical extract, 17 hydroxy 11-dehydrocorticosterone (Compound E) and corticosterone in order of potency, corrected the abnormal carbohydrate metabolism.

PROTOCOL

E. L. (Number 193336) a 51-year-old single business woman, was admitted to The Johns Hopkins Hospital on March 3 1940, complaining of "weakness and sick stomach" for 6 months. She had first noticed loss of strength and weight in 1935 and since then had been troubled with spells of nausea. In November 1938 she had had an attack of bilateral bronchopneumonia necessitating hospitalization for 18 days. Since then she had never recovered her strength. While in the hospital a nurse had commented on the darkness of her skin and she herself had noted increasing pigmentation of the gums, nipples, abdomen and hands. She also had experienced spells of hunger associated with nervousness and sweating which were relieved by food and she had developed the habit of eating fruit or milk between breakfast and lunch. On occasions it was necessary for her to stop on her way home from work late in the afternoon for a sandwich because of extreme hunger.

Her father had suffered from chronic bronchitis and had had an arrested pulmonary tuberculous lesion. She had had malaria in 1901, a partial thyroidectomy in 1914 and typhoid fever in 1919. In 1932 she had had an appendix drained and a subsequent repair of the incision. A cyst had been removed from the left breast in 1934. During the past two years her menstrual periods had become irregular.

Physical examination on admission March 3 1940 revealed an undernourished middle-aged woman who appeared chronically ill. Her temperature was 99° F., pulse rate 92 per minute, respirations 20 per minute and blood pressure 86/70 mm. Hg. There was generalized dark brown pigmentation of the skin, most marked over the elbows, hands, knees and in the axillary folds. There were many small black freckles and the areolae were almost black in color. There was pigmentation of the gums and external genitalia. Some tortuosity of the retinal arteries was noted. The tonsils were small. There was a small amount of thyroid tissue palpable. The lungs were clear. The pulses were of poor volume. The heart was small and the sounds distant. The second pulmonic sound was reduplicated and louder than the aortic second sound. There were no cardiac murmurs. No abdominal organs or masses were felt. Pelvic and rectal examination revealed no abnormality. The neurological examination was normal.

The essential laboratory data were as follows

Hemoglobin 80 per cent (12 grams) red blood cells 3.7 million, hematocrit 33 per cent cells, white blood cells

6,000 with polymorphonuclears 58 per cent, eosinophiles 4 per cent, monocytes 3 per cent and lymphocytes 35 per cent, the sedimentation rate 29 mm. in one hour, corrected. The blood Wassermann was negative. The blood non protein nitrogen was 32 mgm. per 100 cc. and the blood glucose 72 mgm. per 100 cc. On admission she was given intravenous hypertonic sodium chloride. The following morning the serum carbon dioxide combining power was 52.2 volume per cent. Chloride was 109.2 meq per liter sodium 139 meq per liter and potassium 5.1 meq per liter of serum.

The urine was found to have a specific gravity of 1.010 and no albumin or sugar. There were no formed elements. Cultures were negative for acid fast bacilli. A phenolsulfonphthalein test showed a dye excretion of 48 per cent in 2 hours.

X rays revealed fibroid infiltration at the left apex, no adrenal calcification and a normal sella turcica. The basal metabolic rate was minus 15 per cent. Gastric analysis showed no free hydrochloric acid until after histamine when it rose to 40. The electrocardiogram was normal. Venous pressure was 95 mm. of water. Circulation time with sodium cyanide was 18 seconds and with paraldehyde was 8 seconds. Tuberculin tests were negative at a dilution of 1 to 100,000 and 1 to 10,000. Carbohydrate studies are recorded in the text.

Course. During the first month in hospital this patient was maintained on supplementary sodium chloride therapy by mouth (10 grams) and frequent intravenous infusions of sodium chloride and glucose solution. On this regimen she was unable to be up and about for more than very brief periods. Her appetite was poor, her blood pressure 88/50 mm. of Hg and her weight 44.0 kgm. During her course of hospitalization she had one rather severe episode of vomiting and diarrhea, and in addition an urinary tract infection. During these periods she was given desoxycorticosterone acetate and adrenal cortical extract. With the subsidence of the infection the patient was started on a regimen of 5 grams of added sodium chloride by mouth and a single daily intramuscular injection of 4 mgm. of Percorten (desoxy corticosterone acetate in oil). On this regimen her strength improved, she gained 4 kgm. in weight and her blood pressure increased to a level of 130/85 mm. of Hg. On June 23 1940, 8 pellets of crystalline desoxycorticosterone acetate (125 mgm. each) were implanted in the subcutaneous tissues of the left infrascapular region. The daily injections of Percorten were discontinued. The added sodium chloride therapy (5 grams daily) was maintained and the patient was discharged.

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CHOLINE ESTERASE OF BLOOD CELLS AND PLASMA IN BLOOD DYSCRASIAS, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA

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Interest in the distribution of choline esterase activity between the cells and plasma dates back a dozen years or more but relatively little investigation has been carried out. In 1928 Galehr and Plattner (1), using a biological assay method, found that there was more choline esterase activity in human blood cells than in serum and their finding was confirmed by Matthes (2) in 1930. The esterase activity of whole blood, hemolyzed blood and serum of man and other species was studied by Ammon and Voss (3). Human blood, whole or hemolyzed was found to be more active than serum, and dog blood less active than serum. The distribution of esterase activity between blood cells and serum of defibrinated blood was studied by Stedman and Stedman (4) in normal persons and in a number of species of animals, and by Stedman and Russell (5) in twelve myasthenia gravis patients and a group of muscular, neuro muscular and psychopathic cases. They found marked species differences both as to choline esterase activity and as to distribution of activity between plasma and cells. In the normal human they found that the cells have consistently more activity than the plasma. Their myasthenia gravis patients showed a decrease in plasma esterase and an esterase value for cells approximating the normal values. In the myopathic and psychopathic group they found a general lowering of plasma esterase and a variety of disturbances of the esterase distribution.

That the activity of the formed elements of the blood resides chiefly in the red cells is generally accepted. Ginsberg *et al* (6) found that washed pus cells showed no esterase activity. In April, 1940, Alles and Hawes reported qualitative differences between the choline esterase of blood cells and of plasma in their behavior toward different substrates and toward different concentrations of the same substrate. Further reference to this work will be made in the discussion.

Information concerning the esterase activity in anemias is very meager. Antopol *et al* (7) list a case of secondary anemia and one of acute hemolytic anemia in a series of cases of jaundice and liver disease in which there was a general lowering of serum esterase. Milhorat (8) reports values for a long list of varied clinical conditions. Among them are a case of primary anemia and one of acute myeloid leukemia. The degree of anemia is not stated for either case. The primary anemia showed a normal esterase activity in the serum the leukemia, a markedly lowered value. He states that no correlation was found with red cell count or hemoglobin in this series.

As a tentative investigation preliminary to the present study determinations were made on the esterase activity of two dogs in the laboratory of the Pathology Department, through the courtesy of Dr Sidney Madden and Mr Albert Rowe. These dogs were made anemic by repeated phlebotomy. In the normal period the dogs were found to have an appreciably higher esterase activity in the plasma than in the whole blood. This is in accord with the results of Ammon and Voss (3) and with unpublished results of Dr Fenn in this laboratory. As the hematocrit fell, a lowering of the plasma esterase was observed. A slight fall in the whole blood esterase occurred, but the calculated value for blood cells showed a marked rise. On this lead it was decided to investigate the esterase activity of the cells and plasma of patients with anemias of various etiologies.

METHOD

Measurement was made of the rate of production of acetic acid from an excess of acetyl choline substrate by the enzyme present in a measured amount of plasma or whole blood. A continuous titration method was used similar to Glick's modification (9) of the method of Stedman, Stedman and Easson (10) and similar to the

method of Alles and Hawes (11) Blood and plasma were measured in a capillary pipette calibrated to hold 0.202 cc. This amount was delivered into 95 cc of distilled water in a 100 cc. beaker, and the pipette was washed back seven times with this water. The beaker was then placed in a water bath, the temperature of which was kept between 24 and 26° C and as near as possible to 25° C. The temperature of the experimental solution was read at intervals by means of a thermocouple and galvanometer. Into the solution also dipped the end of a microburette, a glass electrode, a glass stirring rod with propeller, run by a special telechron motor at 300 rpm, and a short agar-saline bridge to a small vessel also immersed in the bath, and containing 0.85 per cent NaCl solution and the calomel electrode. The electrodes were led off to a Beckman pH meter by shielded leads. The water bath and electrodes were further shielded by a sheet metal cage with wire door, which also offered some protection from temperature changes.

The salt bridge was necessary because esterase activity is affected by K ions (12). It was found by checking with a standard buffer solution that the salt bridge made the solution read 0.15 pH more acid than its true pH, in the neighborhood of pH 7.50. This difference was found to be constant, and the meter was just as steady with the bridge as without it. A reading of 7.50 was used as the end-point in these titrations, so the true pH was 7.65. This was also checked by comparison with indicators.

After reaching temperature equilibrium, the solution containing esterase was brought to approximately pH 7.6 (as read on the meter) with dilute NaOH, 10 cc. of a 1 per cent solution of acetyl choline chloride (Hoffmann-LaRoche) in M/750 phosphate buffer was added, and enough water to bring the volume to 100 cc. The indicator dial of the meter was kept set at 7.50. The time when the alkalized solution crossed the 7.50 mark was clocked with a stop-watch. Then standardized NaOH approximately 0.02 N was run in from the microburette to maintain the solution between pH 7.48 and 7.52 as read on the meter. At the end of approximately fifteen minutes the solution was made more alkaline, about pH 7.55, and the time again clocked when the needle passed pH 7.50. The rate of hydrolysis was found to be constant over a period of one hour in preliminary experiments. All determinations were done in duplicate.

Temperature corrections were made from a calibration curve determined experimentally. The corrections were never in excess of 1 per cent of the normal rates and 5 per cent for the very low rates. This arrangement was considered satisfactory since the changes reported are large. The difficulty of putting a thermostatted water bath in the cage has made it impracticable to introduce this feature for the present.

Blanks were run on normal and abnormal blood or plasma and buffer, and on acetyl choline with its buffer, and each proved to have a small rate of acidification (the acetyl choline solution slightly greater than the enzyme preparation) amounting to 1 to 2 per cent of the rate usually observed for enzyme-substrate preparations.

Since correction would tend to accentuate rather than diminish the contrasts which are being reported, the figures have not been corrected for this small error.

Blood samples were taken from patients in the hospital and clinic, and from staff members of both sexes and various ages for controls. No attempt was made to take all blood samples at the same time of day, as many of the patients were acutely ill and were treated or transfused very soon after admission, and others were seen in the clinics at various times of day. This is probably of no significance, as it has been shown that no diurnal or prandial cycle is demonstrable (13, 14, 15). Ten cc. of venous blood was taken into two oxalate bottles. Plasma and whole blood for esterase determinations were preserved with thymol, which was shown in preliminary experiments to be without effect on the esterase rate, and placed in the icebox. All samples were run within forty-eight hours, and the observation of others (11, 13, 16) that preservation for several days does not alter the esterase activity has been confirmed. Hematocrit determinations and red cell counts were made in the usual way. Hemoglobin was determined with a Sahli hemoglobinometer. Reticulocytes were counted after staining wet preparations with brilliant cresyl blue.

CALCULATIONS AND DESIGNATIONS

Esterase activity is expressed as cc. of 0.01 N NaOH required to neutralize the acetic acid formed from acetyl choline per minute per cc. of blood, plasma or cells at pH 7.65 and temperature 25° C. This value is given the designation *E*. The cell *E* as measured was, on the average, slightly lower than the calculated cell *E*, and the standard deviation of a number of determinations on a single sample was somewhat greater. The results of Alles and Hawes, which were published after the work reported here was finished, indicate that this may be due to the lower NaCl content of the cell preparations. The whole blood and plasma values are probably more valid than the measured cell values, so the latter have been discarded and only the calculated values used.

Activity of the cells is referred to as cell *E* when activity per cc. of cells is meant. Since the mean corpuscular volumes (MCV) vary considerably under conditions of anemia and reticulocyte response, it was found convenient to express a "mean corpuscular esterase," designated MCE. This value was found by multiplying the cell *E* by the MCV in cc. (or the MCV in cu micra as used clinically $\times 10^{-12}$). For convenience this value is expressed multiplied by 10^{10} .

STATISTICAL TREATMENT

The mean values for six normal persons are given in Table I. No other value was regarded as significantly different from the normal unless this was indicated by the "t test" of Fisher (17) with a probability of at least 98 per cent.

The heterogeneity of the group in regard to

TABLE I

Choline esterase of whole blood, plasma and blood cells of patients with anemia and of normal persons

Case number	Condition	Red blood cells	Hematocrit	Reticulo-cytes	Hemo-globin	Choline esterase activity*			
						Whole blood	Plasma	Cell	M.C.E.† X10 ³
1	Hemorrhage	millions 2.2	per cent 19.8	per cent 6.8	grams 6.1	0.264	0.102	0.919	0.823
2	Hemorrhage	2.0	19.6	12.3	6.3	0.264	0.100	0.944	0.901
3	Leukemia	1.3	13.7	0.2	4.0	0.190	0.099	0.809	0.823
4	Leukemia	1.8	16.1	0.3	4.6	0.216	0.112	0.794	0.695
5	Hodgkin's	2.0	20.3	0.3	7.0	0.184	0.079	0.603	0.603
6	Erythroblastic anemia	1.6	13.3	25.0	2.5	0.184	0.106	0.691	
7	Macrocytic anemia	2.3	24.7	4.4	6.0	0.225	0.081	0.663	0.723
8	Plummer-Vinson syndrome	5.1	34.8	1.5	7.2	0.312	0.148	0.693	0.473
9	Microcytic anemia	4.6	32.3		16.6	0.398	0.177	0.867	0.613
10	Polycythemia vera	5.8	34.5		15.0	0.324	0.171	0.615	0.369
11	Treated pernicious anemia	4.4	45.2		13.3	0.391	0.224	0.593	0.610
12	Treated pernicious anemia	5.0	41.3		14.3	0.254	0.165	0.391	0.321
	Normal	5.7	50.0		15.0	0.407	0.153	0.660	0.577
	Normal	5.7	42.3		14.8	0.343	0.175	0.577	0.430
	Normal	4.1	36.1		13.0	0.319	0.183	0.582	0.494
	Normal	5.2	44.4		14.3	0.341	0.188	0.532	0.452
	Normal	4.9	40.2		13.9	0.333	0.219	0.496	0.422
	Normal	5.7	45.8		14.8	0.273	0.139	0.437	0.371
Average of normals						0.336	0.176	0.547	0.484

* Choline esterase activity (E) is expressed as cc. of 0.01N NaOH required to neutralize the acetic acid formed from acetyl choline per minute per cc. of blood plasma or cells at pH 7.65 and temperature 25°C.

† M.C.E. (the esterase activity per cell) is found by multiplying the cell E by the M.C.V. in cc. This value is expressed multiplied by 10³.

age, sex and nutrition is not considered to be of importance since it has been found that esterase activity of the blood is not affected by age, sex, weight, menstruation, pregnancy, exercise, diet (13), or by fasting (3, 13)

RESULTS

A group of fifteen cases and six normals was studied. Nine cases of anemia other than Addisonian pernicious anemia, one case of polycythemia vera, two cases of adequately treated pernicious anemia, and three cases of severe, practically untreated pernicious anemia which were followed through treatment to partial or complete restoration of the blood picture to normal.

Plasma esterase The values for plasma E were in accord with those reported by others who have found low esterase in serum of patients who were debilitated or comatose from a variety of causes (3, 7). All of the cases with markedly low hematocrit and red count showed low plasma esterase activity. One case of idiopathic hypochromic anemia with Plummer-Vinson syndrome showed a normal plasma esterase, although she

had only 7.2 grams of hemoglobin, with a red count of 5,100,000 and a hematocrit of 34.8 per cent. A microcytic secondary anemia with normal red count and lowered hematocrit showed normal esterase values throughout. The adequately treated pernicious anemia patients had normal or slightly high plasma esterase values. It may be noted that the patient (Case 11) with a slightly high plasma value had marked, long standing combined system disease. No correlation was observed between the degree of lowering of the plasma esterase and the severity of the anemia or degree of debilitation but the absence of correlation is probably not significant because there was an inadequate number of cases and not enough of moderate severity. Only one pernicious anemia patient could be followed through to complete restoration of the blood picture to normal. His plasma esterase gradually rose, to attain a normal value when he was seen in clinic with a hematocrit of 46.6 per cent and a red count of 4,540,000.

Whole blood esterase All of the patients with markedly lowered hematocrit and red cell count showed significantly lowered whole blood

activity, and this was in a general way related to the state of debilitation of the patient. Since the whole blood values represent a combination of plasma and cell esterase, discussion of the cell esterase values will be preferable to discussion of the whole blood values.

Cell esterase and mean corpuscular esterase

There appears to be an increase of the cell *E* and MCE in anemias if the bone marrow is not pathological. This was best observed in two cases of acute hemorrhage from peptic ulcer (Cases 1 and 2), both having the cell *E* approximately 170 per cent of the mean normal value, and the MCE 170 per cent and 190 per cent, respectively, of the mean normal value. Two cases of leukemia also had high esterase in the cells, one chronic lymphoid leukemia (Case 3) and one chronic myeloid leukemia (Case 4). These patients both had severe anemia of long standing without any reticulocyte response at the time they were examined. Both showed cell *E* approximately 150 per cent of the mean normal value. The marrow of both was presumably infiltrated with leukemic cells, and hence not really free of pathology, but insofar as the marrow was able to withstand the mechanical interference, its functioning probably approximated the physiological.

A macrocytic anemia of undetermined etiology (Case 7), not responding to liver therapy, showed a normal cell *E* with a significantly high MCE. A moribund child with erythroblastic anemia (Cooley's) showed a moderate but statistically significant rise in cell *E*. Due to the extreme heterogeneity of the cells, it seemed meaningless to compute an MCE for this case. A moribund young woman (Case 5), diagnosed clinically as aplastic anemia and found at autopsy to be a case of Hodgkin's disease with extensive invasion of the marrow, showed a low plasma *E* and a normal cell *E* and MCE. A case of idiopathic microcytic hypochromic anemia (Case 8) had an elevated cell *E* but a normal MCE. A case of microcytic anemia secondary to long-standing rheumatic infection (Case 9) had normal plasma and whole blood *E*, but markedly elevated cell *E* and slightly elevated MCE. A case of polycythemia vera (Case 10) with normal red count maintained by repeated phlebotomy had normal values throughout. Unfortunately, no case of untreated polycythemia vera was available for

comparison. Of the two adequately treated pernicious anemias, one had normal values throughout, the other had a normal cell *E* and a significant elevation of the MCE.

The values for all of these cases are given in Table I, and the relevant clinical data immediately follow.

*Case 1, Number 81157,*¹ was a fifty-eight year old Italian man, with an ulcer history of fifteen years' duration, but with no acute episodes since 1938. Diagnosis was acute hemorrhage from peptic ulcer. The response to transfusion, iron therapy and smooth diet was satisfactory.

Laboratory data Erythrocytes 2,200,000 Hemoglobin 61 grams Leukocytes 7,100 with normal differential and smear Hematocrit 198 per cent. Reticulocytes 68 per cent. Urine 1+ albumin. Stool tarry, 4+ guaiac, many red and white blood cells. Blood Wassermann negative.

*Case 2, Number 159058,*¹ was a seventy-five year old American white man, first seen in 1939, when diagnosis was acute alcoholism and posterolateral sclerosis. On present admission the patient was brought in by the police, having lost consciousness and fallen. There had been incontinence but no convulsions. There was a history of anorexia during the past few weeks, but tarry stools and vomiting were denied. Admission diagnosis was acute hemorrhage from peptic ulcer, and posterolateral sclerosis. Response to transfusion, iron therapy and smooth diet was satisfactory.

Laboratory data Erythrocytes 2,050,000 Hemoglobin 63 grams Leukocytes 9,800 with normal differential and smear Hematocrit 196 per cent. Reticulocytes 123 per cent. Urine negative. Stool tarry, 4+ guaiac, many red and white blood cells. Blood Wassermann negative.

*Case 3, Number 157791,*¹ a Polish-American man of fifty-seven, was admitted with complaint of progressive weakness, palpitation and dyspnea. Diagnosis was chronic lymphoid leukemia and secondary anemia.

Laboratory data Erythrocytes 1,330,000 Hemoglobin 40 grams Leukocytes 38,000 with 98 per cent lymphocytes. Smear achromia, anisocytosis and poikilocytosis. Hematocrit 137 per cent. Reticulocytes 0.2 per cent. Urine and stool negative. Blood Wassermann negative. Sternal biopsy infiltration of the marrow with lymphoid cells.

*Case 4, Number 53588,*¹ an American white woman of fifty-eight, had a long history in this hospital and clinic, with diagnosis of chronic myeloid leukemia. When seen in clinic, at the time this blood sample was taken, the patient was ambulatory but actually appeared moribund. She was exceedingly pale and scarcely able to walk. The spleen was down to the pelvis, and the liver down

¹ These numbers are the hospitalization numbers of patients at Strong Memorial and Municipal Hospitals, Rochester, N. Y.

1 finger's breadth below costal margin. She was admitted to the hospital, but was discharged against advice and died at home about one month later.

Laboratory data Erythrocytes 1,800,000 Hemoglobin 46 grams. Leukocytes 157,000 with 92 per cent myeloid cells the Schilling count showing a striking shift to the immature types. Hematocrit 16.1 per cent. Reticulocytes 0.3 per cent. Urine and stool negative. Blood Wassermann negative.

Case 5 Number 149568¹ a twenty two year old American negro, was admitted in a prostrated and semi comatose condition. Clinical diagnosis was aplastic anemia, with question of aleukemic leukemia or agranulocytic angina. The patient died a few days after admission, and autopsy showed extensive Hodgkin's disease involving the mediastinal and abdominal lymph nodes and the marrow.

Laboratory data Erythrocytes 2,010,000 Hemoglobin 70 grams. Leukocytes 900 with 90 per cent lymphocytes. Hematocrit 20.3 per cent. Reticulocytes 0.3 per cent. Urine and stool negative. Blood Wassermann negative.

Case 6 Number 57332¹ an eight year old Italian girl with a long history in this hospital was diagnosed in 1937 as erythroblastic anemia. On her last admission the patient was extremely undernourished, pale, irritable and apparently moribund. There was marked cardiac decompensation. The liver was down to the umbilicus, and the spleen to the pelvis. Massive ascites was present, and the viscera could only be felt after paracentesis. The patient died about two weeks after this sample was taken. Discharge diagnosis was erythroblastic anemia, myocardial insufficiency subacute hemolytic streptococcus peritonitis and malnutrition.

Laboratory data Erythrocytes 1,600,000 Hemoglobin 2.5 grams. Leukocytes 29,000 with 78 per cent polymorphonuclears. Smear 50 nucleated red cells per 100 white cells marked anisocytosis, poikilocytosis, fragmentation and basophilia. Hematocrit 13.3 per cent. Reticulocytes 25 per cent. Ascitic fluid 2.06 per cent protein 30 mgm. per cent nonprotein nitrogen. Hemolytic streptococcus on culture. Blood culture negative. X rays of the skull changes typical of erythroblastic anemia. Urine and stool negative. Blood Wassermann negative.

Case 7 Number 55711¹ an Italian woman of fifty eight, was admitted by Emergency complaining of abdominal pain and diarrhea of one week's duration. The skin appeared yellow. The tongue was smooth and atrophic. Admission diagnosis was pernicious anemia. Liver therapy produced no change in the reticulocyte count. A number of studies were carried out, but the patient was discharged to clinic with a diagnosis of macrocytic anemia of unknown etiology, latent syphilis, chronic leukopenia of unknown cause and arteriosclerotic heart disease.

Laboratory data Erythrocytes 2,270,000 Hemoglobin 6.0 grams. Leukocytes 3,300 with normal differential. Smear anisocytosis, poikilocytosis, rare megaloblasts and occasional stippling. Hematocrit 24.7 per cent. Re-

ticulocytes 4.4 per cent. Urine and stool negative. Blood Wassermann positive. Icterus index 11 Gastric analysis no free HCl fasting after alcohol and after histamine.

Case 8 Number 1868¹ an American Jewish woman of forty five, was admitted to the hospital with complaint of pain in the right side, difficulty in swallowing and constipation. The tongue was red and smooth. The finger and toe nails were spoon shaped. The liver was palpable just below the costal margin. Diagnosis was idiopathic hypochromic microcytic anemia with Plummer Vinson syndrome.

Laboratory data Erythrocytes 5,100,000 Hemoglobin 7.2 grams. Leukocytes 5,600 with normal differential and hypochromic smear. Hematocrit 34.8 per cent. Reticulocytes 1.5 per cent. Urine and stool negative. Blood Wassermann negative. Gastric analysis no free HCl fasting after alcohol and after histamine. G.I. series megacolon.

Case 9 Number 163127¹ an English woman of forty four was admitted with polyarthritis of two weeks' duration with a past history of rheumatic fever and arthritis. The heart was markedly enlarged, with systolic murmurs. Diagnosis was acute rheumatic fever rheumatic pleurisy rheumatic heart disease, chronic tonsillitis and secondary anemia.

Laboratory data Erythrocytes 4,570,000 Hemoglobin 16.6 grams. Leukocytes 16,000 with normal differential. Smear marked poikilocytosis, with some cigar shaped cells. Hematocrit 32.3 per cent. Urine and stool negative. Blood Wassermann questionable on repeated examinations spinal fluid Wassermann negative. Sedimentation rate 18 mm. per hour.

Case 10 Number 71716¹ was an American white woman of forty five, with a history of polycythemia vera, mitral insufficiency and epilepsy since 1933. She had been hospitalized on many occasions and treated with phenylhydrazine and by phlebotomy. Her red cell count had been as high as 7,300,000, with 24 grams of hemoglobin. The spleen had been palpable at all times, extending as low as 4 finger's breadth below the costal margin. At the time when this blood sample was taken, the patient was seen in clinic, with red blood cells 5,770,000 hemoglobin 15.0 grams hematocrit 34.5 per cent and reticulocytes 0.8 per cent.

Case 11 Number 162882¹ an Irish American man of fifty a known pernicious anemia case, treated over several years with intramuscular liver had marked neurological symptoms and signs. At the time when this blood sample was taken, the patient was seen in clinic, with red cell count 4,380,000 hemoglobin 13.3 grams and hematocrit 45.2 per cent.

Case 12 Number 53987¹ an American white woman of forty four had a history of pernicious anemia and several bouts of intestinal obstruction. The blood picture had been maintained by liver therapy in the clinic. At

the time of this sample, the red blood cells were 5,030,000, the hemoglobin 14.3 grams, and the hematocrit 41.3 per cent.

Addisonian pernicious anemia

Three patients first seen with severe, untreated or practically untreated pernicious anemia, were studied. One was followed through to complete restoration of the blood picture to normal, one to partial recovery, and the third could be followed for only two weeks.

*Case 13, Number 161475,*¹ an American white man of sixty, was admitted by Emergency on January 23, 1940, in a state of prostration, dyspnea and extreme pallor. For four months prior to admission he had been growing progressively weaker, and had been bed-ridden for one month. Increasing pallor, with recent appearance of a yellow tinge, had been noted. Paresthesias of the hands and feet had been present for two months. A small amount of treatment with liver preparations by mouth had been received, but no intramuscular therapy.

Physical examination. The patient was a well-developed, poorly nourished, thin, weak and exhausted man of sixty, with a lemon-yellow skin and extreme pallor, appearing acutely ill. The temperature was 38° C, the pulse rate 112, the respirations 29, and the blood pressure 108/58. The fundi showed old and new flame-shaped hemorrhages, cotton-wool exudates and very yellow discs. The tongue was smooth and red. The heart was somewhat enlarged, and a systolic murmur was heard. The liver was 2 finger's breadth below the costal margin. Neurological examination was negative except for diminished vibratory sense in the extremities.

Laboratory data. Erythrocytes 1,050,000. Hemoglobin 3.2 grams. Leukocytes 3,500 with normal differential. Smear marked poikilocytosis, anisocytosis and basophilic, many nucleated red cells and some megaloblasts. Hematocrit 11.0 per cent. Reticulocytes 1.2 per cent. Urine and stool negative. Blood Wassermann negative. Icterus index 14. Gastric analysis: no free HCl fasting, after alcohol and after histamine. Electrocardiogram changes compatible with coronary disease.

Course. This patient had a smooth course, with ideal response to treatment. During the first three days the patient received 1 cc. daily of "Reticulogen," and during the remaining twenty-four days of his stay in the hospital he received a total of 2 cc. The blood picture improved steadily and rapidly, as did the clinical condition. Reticulocytes rose to 36 per cent on the fifth day, and gradually fell. On February 20, 1940, he was discharged to clinic. At the time of discharge the red cell count was 3,400,000, and the hemoglobin 9.9 grams. The heart was decreased in size. Improvement continued, the patient receiving 1 cc. of "Reticulogen" intramuscularly once a week in the clinic. When seen on March 12, 1940, the red cell count was 4,540,000, and the hematocrit 46.6 per

cent. Diagnosis was pernicious anemia with subacute combined system disease.

When first seen, on admission, this patient had an amazingly low choline esterase activity of both the blood and plasma. The plasma *E* was 0.058, or 30 per cent of the mean normal value, and the whole blood *E* was 0.083, or 25 per cent of the mean normal value. This is the only case in the series in which the cell *E* and MCE were significantly below normal. Values of 0.278 and 0.292, respectively, or 51 per cent and 60 per cent of the mean normal values, were found. On the fourth day of treatment, when the hematocrit had risen from 11.0 per cent to 22.0 per cent, and the red cell count had risen from 1,050,000 to 1,630,000, the plasma *E* was not significantly altered, but the whole blood *E* was 0.163, double the value on admission, the cell *E* was normal and the MCE significantly above normal. The values for plasma, whole blood and cells continued to rise. By the eighth day the MCE had reached the value of 0.810, 270 per cent of the initial value and 170 per cent of the mean normal value. The MCE leveled off at about this value, reaching 0.856 on the twenty-first day. The cell *E* continued to rise until the twenty-seventh day, when the patient was discharged. The discrepancy between the responses of cell *E* and MCE is directly related to the changing mean corpuscular volume. The esterase per cell reached a maximum fairly early, then, as the cells became more numerous and smaller, the esterase per unit volume of cells became larger without much change in the activity per cell. The whole blood *E* reached a normal value on the twenty-first day and leveled off, rising slightly to the twenty-seventh day. Plasma *E* gradually returned to normal in the same manner, but with some lag behind the whole blood value. This rise to normal with clinical improvement is in accord with the finding of Milhorat (8) in a case of nutritional deficiency and a case of pemphigus vulgaris. When the patient was seen in clinic, six weeks after the beginning of treatment, all values were normal.

*Case 14, Number 163708,*¹ a seventy-nine year old American white woman, was admitted by Emergency on March 31, 1940. No history could be obtained, and the patient had no complaints. Subsequent conversation with

her physician revealed an old history of pernicious anemia treated with "Lextron" orally. He had not seen the patient for three months.

Physical examination The patient was a well-developed, fairly well nourished woman of seventy-nine, disoriented and confused, not appearing acutely ill. The temperature, pulse rate, respirations and blood pressure were normal. The fundi showed mild arteriosclerotic changes. The tongue was smooth and red. The heart was enlarged, with a systolic murmur. The liver was 1 finger's breadth below the costal margin. Lower tendon reflexes were absent; uppers, hyperactive and equal.

Laboratory data Erythrocytes 1,320,000. Hemoglobin 5.0 grams. Leukocytes 3,550 with normal differential. Smear marked anisocytosis, poikilocytosis, a few normoblasts and an occasional megaloblast. Hematocrit 17.4 per cent. Reticulocytes 0.2 per cent. Urine urobilin positive. Stool negative. Blood Wassermann negative. Icterus index 12. Gastric analysis: no free HCl fasting after alcohol and after histamine.

Course On the first day the patient received 0.5 cc. of "Reticlogen" intramuscularly and on the second and fourth days 1 cc. The red cell count and hematocrit rose until the nineteenth day when they leveled off at 3,300,000 and 35 per cent, respectively. Reticulocytes rose to 26 per cent on the fifth day and gradually fell. On the twenty-seventh day 1 cc. of "Reticlogen" was given. No further rise in hematocrit or red cell count was observed during the remainder of the patient's stay in the hospital. She was discharged to the care of her physician on May 9, 1940 with a diagnosis of pernicious anemia, generalized arteriosclerosis, senile dementia and arteriosclerotic heart disease.

When first seen, on admission, this patient had a significantly low plasma and whole blood *E*. On the second day there was a slight rise in the whole blood *E* which was of questionable significance. For some reason, possibly relief of dehydration, the red cell count fell markedly during the first three days. The hematocrit did not change and this would indicate that the MCV changed markedly. The reticulocyte count on this day was 7 per cent, so that active regeneration had begun and the MCV would be expected to rise. However, it seems possible that the red count may have been at fault and this quick esterase response to therapy is open to doubt. On the subsequent days this patient followed much the same course as the first patient, until the nineteenth day, when the blood picture leveled off at 3,500,000 red cells and a hematocrit of 35 per cent. On the nineteenth day the whole blood *E* was normal, the plasma *E* was rising, the

cell *E* and M.C.E. were still high with the M.C.E. leveling off before the other values. On the thirty-first day the whole blood and plasma *E* had fallen, but the whole blood *E* was still within the normal range. Cell *E* and M.C.E. had fallen, but were still significantly high.

Case 15 Number 161604 a sixty-year-old Russian Jewess was admitted by Emergency on January 24, 1940. She had been bedridden for two weeks. Her diet had been very deficient. Recently she had noted the appearance of a yellow color in her skin. She complained of edema of the ankles, and coldness of the hands and feet. She had been treated "for anemia," but apparently had received no liver therapy in any form.

Physical examination The patient was a well-developed, fairly well nourished woman of sixty appearing prostrated, semi-comatose and pale, with a yellow color. The temperature, pulse, respirations and blood pressure were normal. The fundi were pale and showed small hemorrhages. The heart was enlarged, with a systolic murmur. The liver was 2 finger's breadth below the costal margin. Neurological examination was negative.

Laboratory data Erythrocytes 900,000. Hemoglobin 4.4 grams. Leukocytes 5,800 with normal differential. Smear marked poikilocytosis, anisocytosis and basophilic, with some nucleated red cells. Hematocrit 13.0 per cent. Reticulocytes 2.4 per cent. Urine and stool negative. Blood Wassermann negative.

Course During the first six days the patient was given 6 cc. of "Reticlogen" intramuscularly and from this time to the fourteenth day 0.5 cc. daily. Clinical improvement paralleled improvement in the blood picture and the patient was discharged to a nursing home on February 8, 1940 with a red cell count of 2,730,000, 7.9 grams of hemoglobin, and a hematocrit of 29.8 per cent. Discharge diagnosis was pernicious anemia and generalized arteriosclerosis.

When first seen, twelve hours after treatment was started, this patient had esterase values comparable with those of Case 14. During the two weeks of treatment the whole blood *E* rose but did not reach the normal range, the plasma *E* fell but the fall is probably not significant. The cell *E* and M.C.E. were normal at first and rose in a similar fashion to those of the first two patients. A tendency toward leveling off is seen in the M.C.E. values. This case is of little value in itself and merely serves to corroborate the findings on the first two.

Data on the three pernicious anemia patients are given in Table II.

TABLE II

Choline esterase changes occurring during the treatment of pernicious anemia

Case number	Days of treatment	Red blood cells	Hematocrit	Reticulocytes	Choline esterase activity*			
					Whole blood	Plasma	Cell	M C E † × 10 ¹⁰
13	0	1 0	11 0	1 2	0 083	0 058	0 278	0 292
	2			2 1				
	4	1 6	22 0		0 168	0 071	0 513	0 694
	5			36 0				
	7			34 0				
	8	2 0	28 2	14 0	0 211	0 068	0 578	0 810
	10			6 1				
	14			0 4				
	21	3 2	37 5		0 362	0 137	0 738	0 856
	27	3 8	29 8		0 390	0 130	0 808	0 842
14	42	4 5	46 6		0 362	0 199	0 550	0 550
	0	1 3	17 4	0 2	0 128	0 068	0 413	0 500
	2	1 1	17 7	7 3	0 139	0 069	0 463	0 747
	5			25 4				
	7			18 0				
	9	2 0	28 2	18 0	0 253	0 102	0 691	0 927
	12			8 0				
	19	3 5	34 8		0 378	0 111	0 910	0 918
15	12			1 5				
	31	3 5	36 2		0 309	0 085	0 706	0 720
	0	0 9	13 0	2 2	0 127	0 084	0 413	0 595
	6	1 8	23 0	24 3	0 186	0 061	0 578	0 735
	14	2 7	29 8	0 9	0 244	0 056	0 689	0 772

* Choline esterase activity (*E*) is expressed as cc of 0.01N NaOH required to neutralize the acetic acid formed from acetyl choline per minute per cc of blood, plasma or cells at pH 7.65 and temperature 25° C

† M C E (the esterase activity per cell) is found by multiplying the cell *E* by the M C V in cc. This value is expressed multiplied by 10¹⁰

DISCUSSION

There appears to be a mechanism whereby the choline esterase activity and distribution between the cells and plasma are altered under certain conditions of anemia. No change in whole blood or plasma esterase was observed in cases with a hematocrit of more than 30 per cent. Since most of the moderately anemic cases were microcytic, and tended to have normal red cell counts, with low hematocrits, the effect of lowering the red cell count alone cannot be observed in this series. In all cases with hematocrit 25 per cent or less, and with red blood cell count 2,270,000 or less, there was a significant lowering of the esterase values for both plasma and whole blood. The whole blood values, although low, are higher than the values which would be expected if the cell esterase remained normal. There occurs what may be termed, for purposes of discussion, a compensa-

tion for the reduced plasma esterase and the reduced number or total volume of circulating cells. The amount of this compensation was in a general way related to the condition of the patient. The best response was seen in the two acute hemorrhage cases, where the bone marrow was presumably normal and had not been subjected to prolonged drains upon its resources, and in pernicious anemia patients after treatment was started and before restoration of the blood picture was complete. All of these cases showed a good reticulocyte response at the same time as the esterase response. One might naturally suppose that the increase in cell esterase was associated entirely with the appearance of reticulocytes in large numbers in the circulating blood. However, it was seen that the two cases of chronic leukemia, with no rise in reticulocyte count, and with anemia of long standing, showed almost equally high esterase activity of the cells. Unless an increased blood destruction in leukemia is postulated, it is impossible to conclude that the high esterase activity of the cells is related entirely to their youth. It seems more probable that there is some mechanism for maintaining a high esterase in the mature cells under conditions of anemia, and that the mechanism fails in the very debilitated or moribund patient, or when the marrow fails seriously.

An explanation of the changes in cell esterase on a basis of surface area of the cells is untenable. Although several of the cases exhibiting elevated mean corpuscular esterase had large cells, some with very high mean corpuscular volumes, *e.g.*, untreated pernicious anemia, showed very low mean corpuscular esterase. Also a case of microcytic anemia had an elevated mean corpuscular esterase. No correlation existed between M C V. and M C E in this series.

The pernicious anemia patients, before treatment, appeared to be unable to make this compensation. By far the lowest cell esterase and whole blood esterase values were seen in these patients, and the fact that immediately upon treatment these values rose strikingly suggests the possibility that in pernicious anemia there is a specific inability to put out choline esterase in adequate amounts in such cells as the marrow produces.

In Case 14, there was, for some unknown reason, a leveling off in the response of the blood

picture on treatment. It may be that the therapy was inadequate. With this leveling off came a drop in plasma esterase, which had been rising gradually while the blood picture was improving. There was also a fall in the cell *E* and MCE which appears similar to the return to normal in Case 13. There is a possibility, however, that this fall represents the beginning of a reversion to the untreated condition. No definite conclusion can, of course, be drawn from a single sample on a single case, but the suggestion can be made that accompanying the arrest in blood picture improvement there may have been some sort of deficiency or inhibition in the choline esterase mechanism which is responsible for this fall.

With the return to a normal blood picture there is a reduction in the esterase activity of the cells of the pernicious anemia patient to the normal level. The period of recovery lasted about six weeks, or somewhat less than the average life of a normal red blood cell. This reduction to normal might be thought to occur simply as the average age of the circulating cells increased. It appears from the leukemia picture, however, that this is probably not the explanation. And since low serum esterase has been reported in acute hemolytic icterus (7) in the face of massive blood destruction it does not seem probable that blood destruction is the mechanism for removing the esterase from the cells into the plasma. How the reduction is controlled and what the mechanism is whereby cells lose and the plasma gains esterase activity are unexplained.

It seems possible, in some of the cases that the mechanism of the reduction in plasma esterase might be simple dilution. When the plasma values are corrected for dilution occurring with the restoration of the blood volume following hemorrhage in Cases 1 and 2, normal values are obtained. But when such a correction is made in the other cases, where the relative plasma volume was chronically high most of the values are still low. Low values have also been reported in a number of non anemic conditions and it seems improbable that plasma dilution can be the explanation.

The studies of Alles and Hawes (11) indicate that blood cell choline esterase may be qualitatively different from plasma choline esterase. They found that at relatively low substrate concentrations the activity of the cells was far greater

than that of the plasma, but that inhibition of the cell esterase occurred at higher substrate concentrations. This work appeared after the present investigations were completed. Figure 2 in their studies indicates that the substrate concentration chosen was such as to give a maximum of cell esterase activity. They also found a marked effect of NaCl a certain concentration being required for maximum activity. This aspect of the condition of anemias must be investigated although it is known that some of the patients had normal blood chlorides, and there was no clinical reason for supposing that any except possibly the acute hemorrhage cases might have had disturbed NaCl balances. The changes observed in cell esterase are not readily explained on a basis of NaCl effects, and it seems improbable in this particular series where the changes we are most concerned with are marked increases over the normal rather than decreases, that the NaCl factor is a determining one.

A recent paper of Nachmansohn (18) shows that divalent cations have marked effects on the activity of choline esterase. The possible significance of these ions in the anemia choline esterase picture with special reference to Mg^{++} is being investigated in collaboration with the Department of Medicine of this university. Other studies which are planned include investigations on animals of the choline esterase distribution and variations under conditions of anemia and blood destruction studies on the bone marrow esterase, and investigations of the properties of the cells of anemic patients with regard to choline esterase.

SUMMARY

1 Choline esterase determinations were made on whole blood and plasma of normal persons and of patients with various blood dyscrasias and anemias. Values for the cells were calculated.

2. The findings on esterase activity of the plasma were in accord with those of others and consisted of a lowering of plasma esterase in conditions of debilitation, in this instance associated with severe anemia.

3 In patients with anemias other than severe, untreated pernicious anemia who were in fair clinical condition or whose bone marrow was relatively free of pathology there occurred a marked rise in the esterase activity of the cells. This high

level of activity in the cells is thought not to be wholly dependent upon the presence of reticulo-cytes and young cells in the circulating blood

4 The cases of untreated, severe pernicious anemia showed a marked reduction in esterase both of the cells and the plasma. On treatment there was an immediate sharp rise in the cell esterase to a strikingly high value. This high esterase activity was maintained for a time, but gradually fell off to normal as the blood picture was restored to normal. The plasma esterase rose slowly and steadily to normal.

5 It is suggested that some mechanism exists for maintaining a high esterase in the cells in conditions of anemia, that this mechanism fails in severe, untreated pernicious anemia, in extreme debilitation and/or in severe bone marrow failure, and is stimulated or rehabilitated in the pernicious anemia patient on treatment with liver therapy. It is also suggested that some mechanism exists for reducing the high cell esterase to normal with the restoration of the blood picture to normal, and that this restoration is something more than a simple liberation of the enzyme by cell destruction.

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FAT METABOLISM IN DIABETES MELLITUS¹

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Hypotheses of fat metabolism in diabetes mellitus

The development of thought concerning the metabolism in diabetes mellitus since the classical experiments of v Mehring and Minkowska has been formulated into two contrasting theories. Evidence of crucial character bearing upon these theories is still being actively sought for by experimenters, but it appears that the totality of this evidence is not yet sufficient to bring complete conviction to all in one direction or the other.

Briefly, these opposing theories may be stated as follows:

1 Under-utilization hypothesis The major if not the sole, defect in the intermediary metabolism in diabetes mellitus is that the peripheral tissue (*i.e.* chiefly muscle) cannot, either at all or in sufficient measure, oxidize carbohydrate without the catalytic intervention of insulin.

2 Overproduction hypothesis Fatty acids are convertible into carbohydrates by the liver. The function of insulin is to control directly or indirectly the extent of this conversion; its action in the periphery, which is to catalyze the oxidation of carbohydrate, is either nil or of minor importance.

According to the first of these hypotheses the complete diabetic subject must lose all of the energy derivable from carbohydrate and most of that from protein, hence he must fall back upon fats as the chief source of his energy requirements. According to the second hypothesis also there is in the diabetic a profound disturbance of the normal course of fat metabolism in the liver. It therefore becomes necessary to examine the theories of fat metabolism which developed simultaneously with the growth of the above hypotheses.

There are two possible chemical mechanisms by which fats can be utilized in the periphery. These are:

I Fats are utilized directly by the periphery; *i.e.*, oxidation is initiated and completed in the muscles themselves.

II There must be a preliminary partial oxidation in the liver to diffusible substances which are oxidizable by the muscles.

The possibility, discussed later, that both I and II are operative at the same time must also be considered. However, differences of opinion arose over the detailed mechanism of II which were expressed in the alternative hypotheses:

II-A Fats are preliminarily oxidized in the liver to ketone bodies plus a two-carbon compound (acetic acid). In the normal subject these are completely oxidized in the periphery.

II-B Fats are initially converted by the liver to carbohydrates as well as ketones for peripheral utilization.

II-C. Fats are completely converted in the liver to ketone bodies only.

Early in the literature the emphasis was placed upon one of these alternatives (II-A) almost to the complete exclusion of the others. The reasons for this were threefold: the ascendancy of the under-utilization school, the strong position of the Knoop hypothesis of successive beta oxidation of fatty acids, and the development of the hypothesis of obligatory coupling of ketone body-carbohydrate oxidation in the periphery. The relation of these to the problem of fat metabolism in the diabetic will be briefly discussed.

Hypothesis of successive beta oxidation of fatty acids

The original experimental work of Knoop (1) upon which this hypothesis was based was confined to the study of phenyl substituted fatty acids in which the side chain contained 5 carbons or less. When these acids were fed the nature of the phenyl residue excreted clearly showed that these short fatty acids were oxidized at the carbon

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atom which was in the beta position to the terminal carboxyl group. In the case of phenyl valeric acid, the longest fatty acid studied, Knoop limited his conclusion to the statement that there was beta and delta oxidation but asserted nothing about the possible paired splitting off of acetic acid or other oxidized two-carbon compound. Nor did he state that the type of oxidation which he showed for these short fatty acids was a general biological reaction for the oxidation of longer fatty acids.

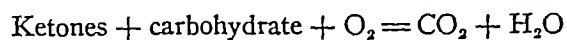
Dakin (2a), however, continued work with phenyl-substituted fatty acids and became convinced that "the evidence obtained" (indicates) "that five acids of the type Ph-CCCCOOH undergo oxidation in the body in such a way that four carbon atoms are removed from the side chain in *two pairs*." This process "may be termed successive beta oxidation" and he saw "no reason to suppose that it is not a general biochemical reaction." For then it became clear "that the catabolism of a fatty acid group, $\text{CH}_3(\text{CH}_2)_n\text{COOH}$, is effected by the successive removal of two carbon groups at a time." It would necessarily follow that each molecule of fatty acid would be degraded through a succession of fatty acids each shorter by two carbon atoms than its immediate precursor. Finally *one* molecule of butyric acid would result which in its turn would be oxidized to *one* molecule of acetoacetic acid or beta hydroxybutyric acid.

Of the nature of the two-carbon compound split off little was known. It was most generally asserted to be acetic acid and as such was incorporated in schemes of fatty acid oxidation. The difficulty, pointed out by Friedmann (3), that acetic acid was markedly ketogenic in perfused livers was generally ignored. For then the fatty acids with odd numbers of carbon atoms, when oxidized by successive beta oxidation, should likewise produce acetic acid and therefore should be ketogenic. But they are not. Dakin (2b), recognizing the difficulty, avoided it by postulating the possible formation of other two-carbon oxidation products of the type of glyoxylic acid. However, nowhere in the literature are there any reports of the isolation or identification of any of these hypothetical two-carbon compounds.

Hypothesis of obligatory coupling of ketone-carbohydrate oxidation

The striking ketone body excretion by the diabetic subject and its equally striking recession following resumption of carbohydrate oxidation remained to be explained. Although there were isolated appeals for a simpler hypothesis (Cf., for example, von Furth (4), Raper and Smith (5)), the hypothesis of obligatory coupled oxidation of ketone bodies and carbohydrates, initially founded upon an aphorism and not much more, dovetailed so neatly with the Knoop hypothesis that it became the almost unchallenged theory of the metabolism of fats in the diabetic for a period of forty years. The statement of this hypothesis as it became fully developed was as follows: the long even-numbered fatty acid chains ($\text{C} \geq 16$) present in natural fats are oxidized by successive beta oxidation in the liver. A two-carbon compound—presumably acetic acid, which is easily oxidized by the peripheral tissues of the complete diabetic—is split off. The fatty acid molecule is thus reduced step by step to the four-carbon butyric acid. This in turn is oxidized to acetoacetic or beta-hydroxybutyric acid which cannot be further utilized by the diabetic and is excreted *in toto*.

If the diabetes is "incomplete" or if insulin is given, carbohydrates can be oxidized and there occurs in the periphery the chemical reaction of coupled ketone body-carbohydrate oxidation, *viz*



This is the sole mechanism by which ketone bodies can be oxidized in normals or diabetics.

The reasons for the ascendancy of this combination of hypotheses are easy to discern.

1 It permitted the under-utilization school of diabetic students to explain how the complete diabetic, who was losing about 70 to 80 per cent of the energy from protein as carbohydrate or ketone bodies, and 100 per cent of that from carbohydrate, could still exist. The calculations in Table I show that there was still a possible 27 to 62 per cent of the energy of the original fat in the form of a hypothetical two-carbon compound split off by the beta oxidation available for the energy requirements of the periphery. The balance was lost to the periphery either in the process of pre-

TABLE I

The heats of combustion of oxidized derivatives of fatty acids and the calculated energy available to the periphery

1 gram mole of fat (triglyceride) = 870 grams = 8200 calories (typical fatty acid = $C_{18}H_{34}O_2$, palmitic)

Substrate formed in liver by partial oxidation of fatty acid	Calories/Mole of fat	Moles/Mole of fat	Total fat calories available to periphery	Gluco-genic
			per cent	
Acetic acid	220	3X6	18	No
Acetaldehyde	251	3X6*	18	No
Glyoxalic acid	167	3X6*	18	See footnote
Glyoxalaldehyde	7	3X6	18	See footnote
Glyoxylic acid	1.5	3X6	18	See footnote
Formic	63	3X13	39	No
Beta-hydroxybutyric acid	438	3X17	51	No
Acetoacetic acid	7	3X17	51	No
Glucose	673	3X2.67†	53	Yes

* By successive beta oxidation hypothesis 1 mole fatty acid = 1 mole ketone + 6 mole substrate

† By multiple alternate oxidation hypothesis 1 mole fatty acid = 4 mole substrate

‡ Hypothetical maximum yield per mole fat.

§ No information found that these compounds are intermediates in fat catabolism. None have been clearly demonstrated to be gluco-genic.

limentary oxidations in the liver or in the form of unoxidizable ketone bodies. The position of the complete diabetic was indeed quite precarious but it was conceivable that his energy requirements could be met by such a mechanism. Curiously enough, as is seen from the table, the formation of acetic acid or glyoxylic acid, which are those most frequently advocated would yield the least amount (27 to 46 per cent) of the energy of the original fat to the periphery.

2 It allowed this school to avoid postulating the formation of carbohydrate from fats, since a compound other than carbohydrate, but presumably oxidizable by the diabetic, was formed in the liver.

3 It allowed of a ready explanation, which appeared to be quantitative of the marked influence of carbohydrate utilization upon urinary ketone body excretion.

The position of the over productionists was less clear. Obviously they could hardly espouse the obligatory coupling hypothesis for then ketonuria would never occur, since by assumption abundance of carbohydrate is oxidized in the periphery. It was natural, then that they concerned themselves with experiments designed to show that the ketone bodies were oxidized in the periphery without coupling with carbohydrate oxidation (6, 7). The

abnormal fat metabolism in diabetes could more easily be explained by postulating that there occurred in the liver the reaction

Fatty acids + oxygen = ketone bodies + glucose + (two-carbon compound?)

The exact stoichiometric proportions of this reaction were never clearly defined. The important assumption was made that this reaction was controlled directly or indirectly by insulin. In diabetes mellitus therefore it ran to excess, resulting in 'over production' of ketone bodies and glucose. The effect of what the under utilizationists call a 'resumption of carbohydrate oxidation in the periphery' in causing a recession of ketonuria and glycosuria could just as readily be explained by asserting that under the same conditions the above reaction was inhibited. However, despite the fact that the hypothesis of successive beta oxidation was the accepted explanation of the mechanism of fat oxidation in the liver certain of its implications were ignored, viz

1 All the conceivable two-carbon oxidized compounds derivable from fatty acids (Table I) have either been shown to be agluco-genic or else have never been shown to be intermediates in the catabolism of fat. It was necessary to fall back upon the assumption that the four-carbon residues of the oxidation of fatty acids were the immediate precursors of glucose, although there was no conclusive evidence that this was so. Butyric acid itself which is known to be gluco-genic in the liver (8b), has never been shown to be an intermediate in the hepatic catabolism of fat. Nor is there any evidence that the ketone bodies themselves are gluco-genic. The recent experiments of Weil-Malherbe (9) showing that acetoacetic acid is converted into glucose by the kidney have not been confirmed (10).

2 Aside from this there is another difficulty if only the residual four-carbon compounds of fatty acid are convertible to glucose. For then both the ketone bodies and glucose originating from fatty acids must come entirely from these residues and the maximum grams (G) of glucose which could be obtained from the catabolism of

F grams of fat when K grams of beta-hydroxybutyric acid are excreted

$$\frac{G}{180} = \left(\frac{F}{870} \times 3 \times \frac{4}{6} \right) - \left(\frac{4}{6} \times \frac{K}{104} \right)$$

$$\text{or } G = 0.41 F - 1.15 K$$

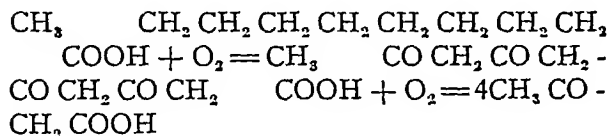
In other words, there should be in the diabetic an *inverse* relation between glucose and ketone excretion, and in the limit, according to the equation, glucose excretion *from fats* should be zero when the grams of urinary ketones is approximately $\frac{1}{3}$ of the grams of total fat catabolized. But no such relations have ever been remotely suggested in innumerable metabolic studies of diabetes mellitus. These considerations logically require that the over-productionists abandon the hypothesis of successive beta oxidation of fatty acids as incompatible with their own views and propose another, although to date no convincing substitute has been suggested.

Evidence against the combined hypotheses

A retrospect of the literature shows that, long before outspoken doubts began to appear, there was evidence which was completely at variance with the successive beta oxidation and obligatory coupling hypotheses when these were carried to their logical conclusions. For example, it was long known that the partition of fats in the livers of fasting normal animals who must be subsisting largely on fats failed to reveal any of the lower fatty acids which were supposed to be formed by degradation of the higher ones. The major portion of the fatty acids contained carbon atoms ≥ 16 . Small amounts (1 to 2 per cent) of myristic (C_{14}) and lauric (C_{12}) were found, but essentially no acids with fewer carbon atoms. In particular, butyric acid should have been formed in large amounts by the livers of diabetic subjects with severe ketosis and, being freely diffusible, should have appeared in the urine. There, as Hurlley (11) pointed out, its presence should long ago have been revealed by its odor alone.² Still more convincing was the evidence in the working diabetic, particularly the exercising de-

pancreatized animal.³ Since these animals were presumably oxidizing no carbohydrate, they should oxidize no ketone bodies. Hence, during exercise, the excess ketone body excretion over the basal excretion could easily be calculated from the excess fat metabolism. But there was no doubt, as found by many observers, that there was essentially no increase of ketone body excretion during exercise despite large increases of fat metabolism. This is clear indication that large amounts of fats were completely utilized without coupling with carbohydrate oxidation. This one fact alone was completely at variance with the obligatory coupling hypothesis and should have forced its abandonment at once.

The more systematic attack upon the Knoop hypothesis may be said to have begun with Hurlley's paper (11). The absence of the lower fatty acids in the livers of diabetics dying of coma led him to reject the Knoop hypothesis and propose instead the hypothesis which became known as the multiple alternate oxidation hypothesis (II-C). According to this, fatty acids are oxidized simultaneously along the whole length of the carbon chain at alternate carbon atoms with complete disruption into ketone bodies according to the scheme



A typical fatty acid such as palmitic ($C_{16}H_{32}O_2$) would accordingly yield not one but four molecules of ketone bodies. Jowett and Quastel (13) determined ketone body formation by liver slices in the presence of various fatty acids. The yield of ketone bodies from the higher fatty acids (C_8 - C_{10}) in comparison to that from the lower ones brought them to the conclusion that the oxidation went by way of multiple alternate oxidation. Deuel, Hallman, Butts, and Murray (14) fed the ethyl esters of fatty acids up to C_{18} to fasting rats. Comparison of the rates of excretion of ketone bodies makes it appear unlikely that successive beta oxidation alone is responsible for the degradation of the fatty acids. For example, in the case

² Butyric acid at pH 5 to 7.4 is easily detectable by its odor at 0.01 M. This is approximately $\frac{1}{20}$ of the urinary concentration of ketone bodies frequently observed in severe ketosis.

³ See for example Barker's (12) recent experiments on exercising depancreatized dogs.

of caprylic acid (C_8), they concluded that delta oxidation as well as beta oxidation occurred so that two acetoacetic acid molecules would result. In the case of palmitic and stearic acids their results were more difficult of interpretation but they concluded that three or possibly four acetoacetic acid molecules might result from one molecule of fatty acid. Their conclusion was that in the case of the higher fatty acids (C_8 - C_{18}) their oxidation proceeded according to the hypothesis of multiple alternate oxidation.

Bluxenkrone-Möller (8a) perfused the livers of diabetic cats and compared the total oxygen consumption with the ketone formation. He found that it was possible to explain the low oxygen to ketone ratio only by assuming that four molecules of ketones were formed per molecule of fatty acid oxidized. Stadie Zapp and Lukens (15a) studied the formation *in vitro* of ketone bodies by slices from the livers of diabetic cats. They compared the experimentally observed ratio of oxygen uptake to ketone formation with the theoretical ones calculated according to the Knoop hypothesis and the multiple alternate oxidation hypothesis. They found (Table II) that, when oxygen uptake

Nevertheless in slices from the livers of diabetic cats they could find no trace of acetic acid formation although the ketone body formation was large. In addition, they found that with liver slices actively producing ketone bodies the molecular ratio of ketone body increase to fatty acid decrease was in accordance with the hypothesis of multiple alternate oxidation rather than that of successive beta oxidation (10).

Present position of the successive beta oxidation hypothesis

The totality of the evidence cited above appears to be convincing proof that the major portion of fatty acid oxidation in the liver occurs in such a way that the entire fatty acid molecule is completely oxidized to ketone bodies. It is not intended to imply that beta oxidation does not occur. Indeed, the original experiments of Knoop and of Dakin are strong proofs that it does. More recently Stetten and Schoenheimer (16), using deuterium have shown that palmitic acid (C_{16}) can be degraded to a small extent step-wise to lauric (C_{12}) and myristic (C_{14}) acids. The point to be emphasized is that the original implication of the successive beta oxidation hypothesis namely that large amounts of intermediary fatty acids down to acetic acid are formed in the liver by oxidation of the long-chain fatty acids, is no longer in conformity with the experimental facts. Successive beta oxidation if it occurs must account for only a small part of the total fatty acid oxidation in the liver most of it appears to be accounted for by multiple alternate oxidation.

Concerning the mechanism by which multiple alternate oxidation is brought about, nothing can be said. It is worth while remarking however, that the conception that there is an enzyme which will "fit" a large triglyceride molecule and cause its complete disruption into ketone bodies without the formation of intermediates is not *a priori* improbable. An analogous case would be that of glycogen whose molecule is much larger (16-18 glucose residues) and yet is rapidly broken down to glucose 1 phosphate by a specific enzyme without the formation of intermediary polysaccharides (17).

TABLE II

The calculated and observed ratios of hepatic oxygen uptake to ketone formation in the diabetic animal (15a)

Hypothesis	Equation for oxidation of a typical higher fatty acid (palmitic)	Ratio O_2 ketones
Knoop	$C_{16}H_{32}O_2 + 6O_2 = C_8H_{16}O_4 + 6CH_3COOH$	6 1
Multiple alternate oxidation	$C_{16}H_{32}O_2 + 50O_2 = 4C_4H_8O_2$	1 25 1
Observed in 6 diabetic cats		1 1 ± 0.12

was corrected for carbon dioxide formation and for the oxygen required for the deamination of amino acids, the ratio did not differ significantly from that calculated by the latter hypothesis. Stadie, Zapp and Lukens (15b) added further evidence at variance with the hypothesis of successive beta oxidation which predicts that some oxidized two-carbon compound presumably acetic acid should be formed from the higher fatty acids. Acetic acid formation should be approximately six times the ketone body formation

The production of carbohydrates from fats by the liver

The conversion of fatty acids into carbohydrates by the liver would supply the muscles with an oxidizable derivative of fat other than ketone bodies. The evidence pro and con has been recently reviewed by Mitchell (18) and Soskin (19). Evidence in favor of this hypothesis has been, as a rule, indirect and circumstantial. Such direct proof as is in the literature has usually failed of confirmation. Stadie, Zapp, and Lukens (15a) moreover, showed that this conversion did not occur in the livers of diabetic cats. With liver slices they found that the summation of the respirations for the main oxidative process occurring in the liver was essentially equal to the total observed oxygen uptake.

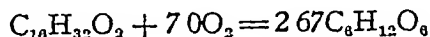
TABLE III

The oxidative metabolism of liver slices from 6 depancreatized cats (15a)

Oxidative process	Mean oxygen uptake per gram liver per hour <i>micromoles</i>
Deamination of amino acids	8.2 ± 1.2
Carbon dioxide formation	28.0 ± 5.0
Ketone body formation	63.0 ± 4.0
Sum of known oxidations	99.2 ± 6.5
Total observed oxygen uptake	87.5 ± 4.0
Difference unaccounted for	-11.7 ± 7.6*

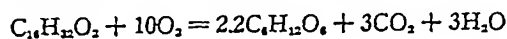
* Difference not significant

It is obvious that fatty acid (*e.g.* palmitic) requires oxygen for its conversion to carbohydrate. The *maximum** yield would be according to the equation



Thus 1 μM of O_2 would suffice for the production of 0.38 μM of glucose. Now, the average excretion of glucose in Stadie, Zapp, and Lukens' series of cats (1 to 4 hours before the actual experiment with the liver slices) was $1100 \pm 167 \mu\text{M}$ per

* It is frequently stated in the literature, apparently upon entirely hypothetical grounds, that the reaction for this conversion has a respiratory quotient of 0.3, *vis.*,



Then 1 mole of oxygen would be equivalent to only 0.22 mole of glucose. The difficulty in balancing the oxygen uptake in the liver against the hypothetical glucose formation would become even greater

kgm cat per hour. Or, since the average weight of the liver is 30 grams per kgm, there would be required approximately 97 μM of oxygen per grams of liver per hour to produce this amount of glucose from fatty acids. *But there was no oxygen whatever available in the metabolism of the diabetic liver slice for this conversion.* This experiment alone, even if there were no others available, is convincing proof that the conversion of fatty acids to glucose does not occur in the diabetic liver. In the case of fasted phlorhizinized rats in which there was marked glucose and ketone-body excretion, they found the same oxygen balance with liver slices as in the diabetic cats (10). They also showed by direct analysis that there was no gluconeogenesis attributable to fat by liver slices from diabetic cats (10).

Direct utilization of ketone bodies by the diabetic

The above discussion makes it clear that the chief oxidation products of fatty acids in the liver are the ketone bodies. The calculations in Table I show that more than 70 per cent of the original energy of fat still resides in these ketone bodies and the question arises as to whether this energy is available to the peripheral tissues of the diabetic. The evidence for the answer that the diabetic can abundantly utilize ketone bodies in the periphery is completely convincing. Stadie, Zapp, and Lukens (15a) reviewed the evidence on this problem and added their own. Table IV gives calculations from data in the literature of the utilization of ketone bodies by the peripheral tissues of normal and diabetic animals. An inspection of this table shows the following

1 Under basal conditions the diabetic animal can utilize sufficient ketones to furnish a large part, if not the whole, basal energy requirements.

2 The potential capacity for utilization under conditions of (a) work or (b) with high blood ketone concentration (induced by injections of ketones) is 4 to 6 times the basal utilization

In other words, it is apparent that, provided the production of ketones from fatty acids by partial oxidation in the liver is sufficient, the diabetic could subsist practically entirely upon ketone bodies, even under conditions of severe exercise. Furthermore, this oxidation of ketone bodies presumably is completely independent of carbohydrate

TABLE IV

Calculations from data in the literature of ketone body utilization by the peripheral tissues of the normal and diabetic animal

	Mean ketone body utilization per kgm. per day mmoles
Charkoff and Soslan (6)	
By measurement of rate of disappearance of injected ketones from blood of	
Diabetic eviscerated dogs	29 ± 1.0
Normal eviscerated dogs	34 ± 5.5
Friedemann (20)	
By measurement of ketone body excretion in eviscerated dogs when injected with very large amounts of acetoacetate	Maximum 120*
Blixenkrone-Møller (8)	
By comparison of ketone formation of perfused diabetic cat livers with prior ketone excretion	39 ± 8.0
By measurement of rate of disappearance of ketone bodies from blood perfused through	
Resting hind limbs of normal and diabetic cats	27 ± 3.6
Working hind limbs of normal and diabetic cats	130 ± 6.7*
Dye and Chidsey (21)	
Injection of acetoacetate at high rate into nephrectomized-depancreatized dogs	Maximum 180*
Stadie Zapp and Lukens (15a)	
(1) By comparison of ketone formation by diabetic cat liver slices with prior urinary ketone body excretion	27 ± 3.6
(2) By measurement of ketone body utilization of normal and diabetic muscle mince in presence of added acetoacetate	22 ± 6.3
(3) By measurement of ketone body utilization by diabetic cat muscle mince simultaneously equilibrated with diabetic cat liver slice	50 ± 10.0
(4) By measurement of the portal hepatic ketone body difference of diabetic cats	30 ± 9.8
Mean basal ketone utilization	32 ± 3.1
Equivalent in grams of fat	2.3 ± 0.2

* Excluded from basal mean.

oxidation. The hypothesis of obligatory coupling of ketone body-carbohydrate oxidation becomes therefore, not only unnecessary but indeed difficult, if not impossible, of retention.

The calculation of the ketogenic-antiketogenic ratio as support of the obligatory coupling hypothesis

There still remained in the minds of the students of diabetes the possibility that there is a definite molecular ratio between carbohydrate oxidized and ketone bodies oxidized. This idea

stemmed from numerous calculations in the literature of the so-called ketogenic-antiketogenic ratio in diabetes mellitus. It remains therefore, to re-examine the calculations and the assumptions upon which they are based.

The assumptions are clearly stated by Shaffer (22). The hypothesis states that antiketogenesis in the human subject is based upon a

ketolytic reaction in the body between acetoacetic acid, the first formed of the acetone bodies and a derivative of glucose (or of other antiketogenic substances), the compound being further oxidized but that failing to react with ketolytic substance, acetoacetic acid is resistant to oxidation accumulates and is excreted. The fact that one finds at the threshold of ketosis an approximately constant ratio between the number of molecules of the precursors of acetoacetic acid and of glucose in the metabolic mixture must mean that the further oxidation of acetoacetic acid constantly taking place under normal conditions is accomplished through a chemical reaction with a derivative of glucose.

The best way to test the hypothesis by means of the quantitative data which are available is to set it up in the form of an equation. It has been frequently emphasized in the literature that the interrelations of ketones and carbohydrates in the intermediary metabolism of the diabetic can only be properly evaluated if the amounts of fat, protein, and carbohydrate in the metabolic mixture are determined by calorimetric methods. Let the partition of the metabolic mixture be

K = Total mM of ketone bodies formed from total fat and protein catabolism. (The ketone bodies derived from fat are calculated assuming 1 molecule of ketone body per mole of fatty acid.)

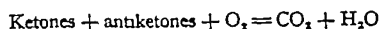
U = mM of ketone bodies utilized.

A = mM of antiketones oxidized

E = mM of ketone bodies excreted

r = 'ketogenic-antiketogenic' ratio a small whole number (1 or 2)

According to the hypothesis there occurs in the periphery as the sole mechanism by which ketones can be utilized the reaction



The total number of ketone body molecules formed in the liver by partial oxidation of fatty acids which can be utilized in the peripheral tissues of either the normal or the diabetic subject can never exceed $r \times A$ mM. Accordingly, if ketone bodies are produced in excess of this value they will be excreted. These relations are expressed by the equation

$$U = K - E = r \times A$$

There are three ways in which the data obtained from subjects with diabetes mellitus can be applied to the equation

1 By the selection of conditions where E is just a little more than zero. Then it may be assumed (practically) that

$$K = r \times A$$

and r may be calculated when K and A are known from the metabolic mixture. This method was called the "measurement of the ketogenic-anti-ketogenic ratio at the ketone body threshold." However, the definition of "ketone threshold" is somewhat arbitrary and the values of r obtained by different observers using this method or by the same observer in different cases were found variant. This was explained away by supposing an "unequal distribution" of metabolites so that in some places glucose molecules were oxidized without encountering ketone bodies and hence were "wasted" as ketolytic agents, while in other cells or localities ketone bodies formed without the possibility of encountering glucose molecules and hence accumulated.

2 Patients with definite excess of K , as indicated by marked ketonuria, were selected, and the observed ketone body excretion compared with that calculated by the equation (r being assigned a value of 1 or 2)

$$E = K - r \times A$$

Unfortunately E is the small difference of two large numbers. Hence the approximate agreement of the small observed and calculated E values was considered sufficient even though, as was frequently found, they differed from each other by several hundred per cent.

3 In patients with definite excess of K the statistics of the equation

$$U = r \times A \quad (1)$$

can be calculated. This is by far the best method since the statistical calculations would constitute an objective test of the equation and the hypothesis upon which it is based. Oddly enough, it appears never to have been used.

Fortunately, there is in the literature a sufficient amount of the necessary data for re-testing this hypothesis in subjects with diabetes mellitus. The cases are all classical ones, reported in the literature before the advent of insulin when marked ketonuria was, of necessity, a frequent accompaniment of the disease. Similar data on human diabetics will in all probability never be obtained again, since it is unlikely that patients with marked ketonuria will be allowed to remain untreated over long periods of time.

The assumptions made in calculating the data are

1 The metabolic mixture as represented by the calories of protein, fat and carbohydrate calculated by the original authors is assumed to be correct.

2 The conversion of calories into ketones and "antiketones" is made by the use of the factors given in Table V.

3 In testing the obligatory coupling hypothesis as expressed by equation (1), the original assumption that one fatty acid molecule yielded one ketone body molecule was adhered to. The employment of the 4:1 ratio as required by the multiple alternate oxidation hypothesis, however, would make no difference in the conclusions drawn from the analysis of the data.

TABLE V

Factors converting calories of metabolic mixture into mM of ketones and "antiketones"

Kgm calories of	Factor	
	Ketogenic	Antiketogenic
Fat on basis of 1:1 ratio	0.363	0.121
Fat on basis of 4:1 ratio	1.45	
Protein	0.442	1.25
Carbohydrate		2.96

Factor converting mM of ketone bodies into grams of original fat = 0.0726 (on basis of 4 moles of ketone per mole of fatty acids) (Average molecular weight of fat = 870)

Presentation of data

1 *Cases of diabetes mellitus with marked ketonuria (i.e. > 10 mM/day)* In this type of case, since there is an excess of ketone forma-

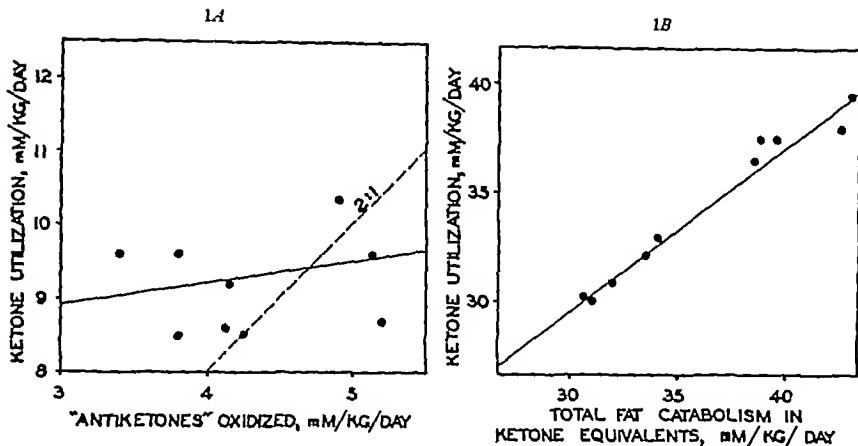


FIG. 1. DIABETES MELLITUS, CASE No. 740 (JOSLIN, 1915)

A Ketone utilization as a function of "antiketones" oxidized. Correlation $\approx 0.27 \pm 0.17$, K/A ratio $\approx 0.27 \pm 0.30$ intercept constant $\approx 8.9 \pm 0.5$ mM/kg/day

B Ketone utilization as a function of total fat catabolism. Correlation $\approx 0.99 \pm 0.04$, $k = 0.75 \pm 0.04$, $U_0 \approx 28.0 \pm 2.1$ mM/kg/day

tion, it is possible to determine whether the data are in accord with the equation

$$U = K - E = r \times A$$

In order to present the data briefly the observations have been plotted for each individual case. The statistical analyses accompany each plot and for convenience of comparison these statistics are collected in a summarizing table

Correlation coefficients, regression coefficients, etc., together with their standard errors are calculated according to standard methods (Cf. Dunn H. A., *Physiol. Rev.*, 1929, 9, 215)

Case number 740 Diabetes mellitus (23) (Figure 1A) Moderate ketonuria ($\approx > 5$ grams beta hydroxybutyric acid per day) The dashed line is calculated by the equation $U = 2A$. The agreement of this line with the observed points is specious since the correlation coefficient is 0.27 ± 0.17 (not significant) and the value of $r \approx 0.27 \pm 0.30$ (i.e. without significant relation to the hypothetical value of 2) Moreover U when $A \approx 0$ (i.e. when no antiketones are oxidized) is 8.9 ± 0.5 mM. per kgm. per day instead of 0. The hypothesis gains no support from this case.

Bessie B Diabetes mellitus (24) (Figure 2A) Ketonuria slight to moderate (beta hydroxybutyric acid 1 to 17 grams per day) The dotted line is theoretical for

$U = 2A$. It bears no significant relation to the observed points. There is no significant correlation (coefficient $\approx -0.40 \pm 0.30$) and the statistical (heavy) line shows a negative value of -0.43 ± 0.35 instead of a value of 1 or 2. There is an appreciable utilization of ketones (13.8 ± 17 mM per kgm. per day) when the "antiketones" oxidized are zero.

Kramer Diabetes mellitus (22) (Figure 3A) Severe ketonuria (28 to 102 grams per day of beta hydroxybutyric acid) There is no significant correlation (-0.10 ± 0.29) The theoretical lines for $U = A$ and $U = 2A$ have no significant relation to the observed points. The statistically calculated value of r is 0.10 ± 0.29 and has no relation to the hypothesis.

Cyril K Diabetes mellitus (25) (Figure 4A) Moderate to severe ketonuria (beta hydroxybutyric acid ≈ 11 to 88 grams per day) Of all the cases in this group the dotted line for the theory $U = 2A$ shows the best apparent agreement to the "eye." But this agreement is found to be specious when the statistical analysis is considered. For then the true equation for the observations is found to be

$$U = 5.6 \pm 1.2 = (0.72 \pm 0.39)A$$

rather than

$$U = 0 + 2A$$

The statistical value of $r = 0.72 \pm 0.39$ is significantly different from the supposed value of 2. Moreover U should be 0 when A (antiketones oxidized) is 0. But it

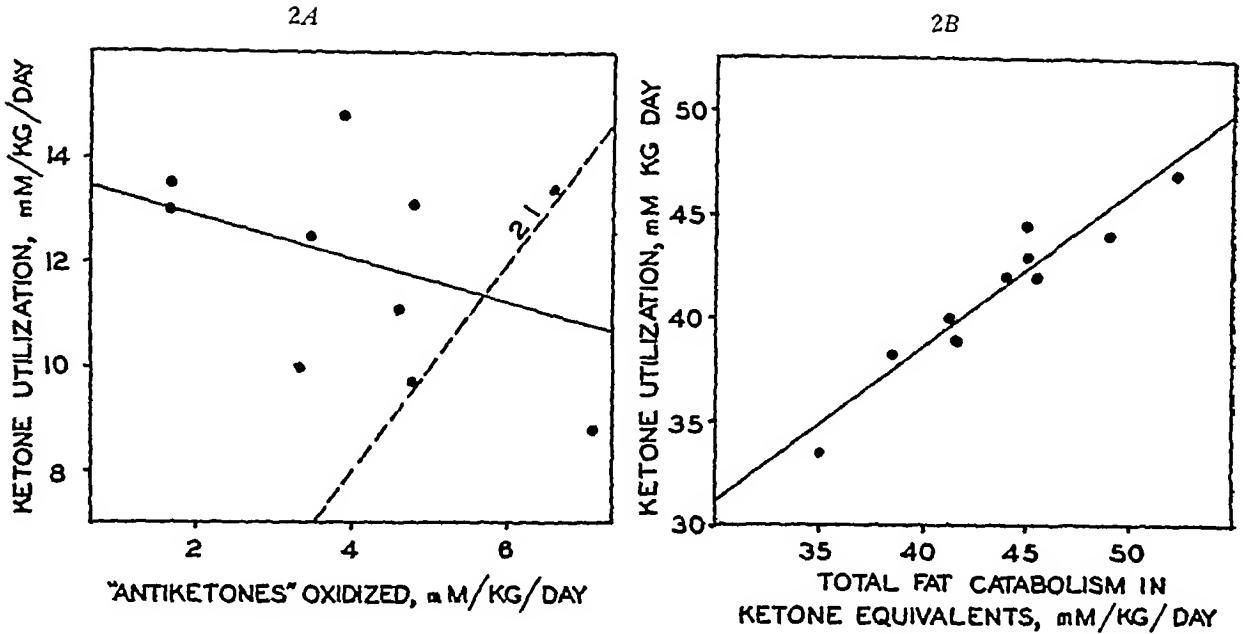


FIG 2 DIABETES MELLITUS, BESSIE B (WILDER, BOOTHBY, AND BEELER, 1922)

A Ketone utilization as a function of "antiketones" oxidized. Correlation 0.40 ± 0.30 , K/A ratio $= -0.43 \pm 0.35$, intercept constant $= 138 \pm 17$ mM/kg/day

B Ketone utilization as a function of total fat catabolism. Correlation 0.95 ± 0.03 , $k = 0.75 \pm 0.09$, $U_0 = 34.5 \pm 4.6$ mM/kg/day

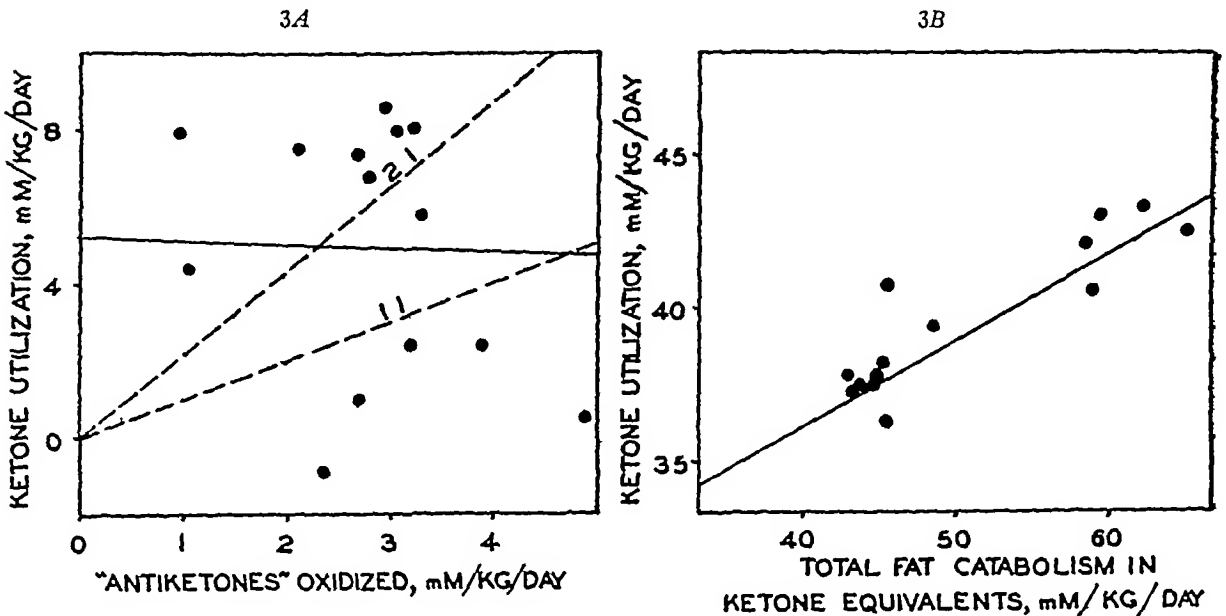


FIG 3 DIABETES MELLITUS, KRAMER (SHAFFER, 1922)

A Ketone utilization as a function of "antiketones" oxidized. Correlation $= -0.10 \pm 0.29$, K/A ratio $= -0.10 \pm 0.29$, intercept constant $= 5.3 \pm 3.1$ mM/kg/day

B Ketone utilization as a function of total fat catabolism. Correlation $= 0.91 \pm 0.05$, $k = 0.28 \pm 0.04$, $U_0 = 34.5 \pm 1.4$ mM/kg/day

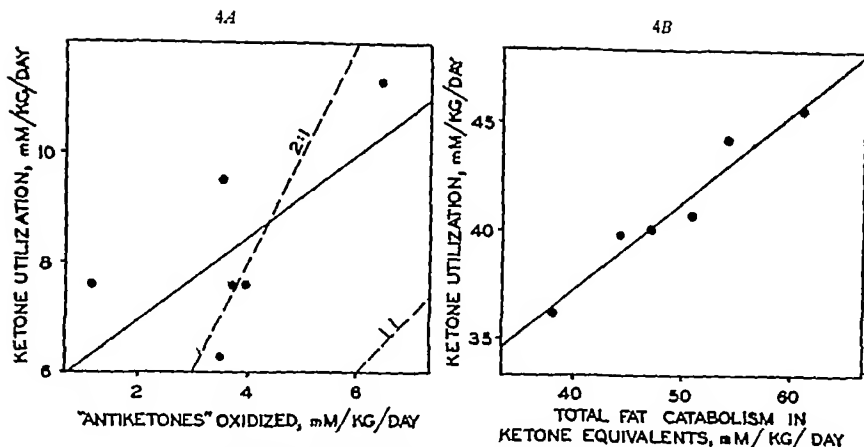


FIG. 4 DIABETES MELLITUS CYRIL K. (GEPHART AUB, DU BOIS AND LUSK, 1917)

A Ketone utilization as a function of "antiketones" oxidized. Correlation = 0.67 ± 0.27 K/A ratio = 0.72 ± 0.39 intercept constant = 5.6 ± 1.2 mm./kg./day

B Ketone utilization as a function of total fat catabolism. Correlation = 0.97 ± 0.03 $k = 41 \pm 0.05$ $U_0 = 35.4 \pm 1.3$ mm./kg./day

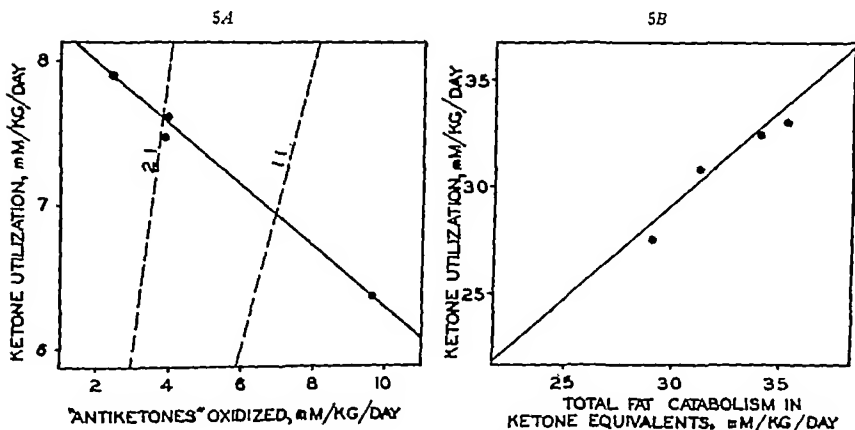


FIG. 5 DIABETES MELLITUS JERVIS B (RICHARDSON AND LADD, 1923)

A Ketone utilization as a function of "antiketones" oxidized. Correlation = -0.99 ± 0.01 K/A ratio = -0.21 ± 0.05 intercept constant = 12.3 ± 0.1 mm./kg./day

B Ketone utilization as a function of total fat catabolism. Correlation = 0.97 ± 0.04 $k = 0.84 \pm 0.14$ $U_0 = 23.2 \pm 1.9$ mm./kg./day

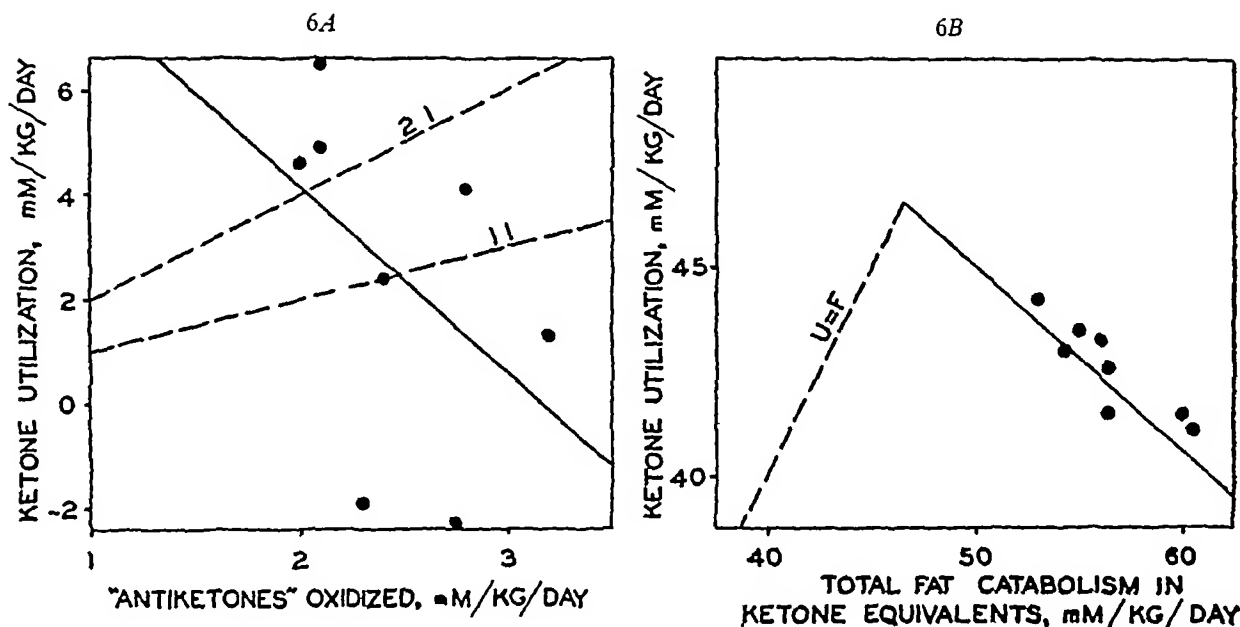


FIG 6 DIABETES MELLITUS, E. W (MOSENTHAL AND LEWIS, 1917)

A Ketone utilization as a function of "antiketones" oxidized Correlation $= -0.45 \pm 0.33$, K/A ratio $= -3.6 \pm 2.4$, intercept constant $= 11.3 \pm 0.8$ mM/kg/day

B Ketone utilization as a function of total fat catabolism. Correlation $= -0.89 \pm 0.08$, $k = -0.38 \pm 0.08$, $U_0 = 46.6 \pm 3.5$ mM/kg/day

is found to be 5.6 ± 1.2 mM per kgm per day. In other words, it is practically certain (prob $= > 0.999$) that there is utilization of ketones when there is no oxidation of "antiketones". The observations in this case, therefore, do not support the hypothesis.

Jervis B, Diabetes mellitus (26), (Figure 5A) Mild ketonuria (2 to 12 grams per day). There is no significant relation between the observed points and the dashed lines calculated for $r=1$ or 2 . There is a negative correlation (-0.99 ± 0.01). The statistical line shows a greater utilization of fat as the carbohydrate oxidation ("antiketones") is diminished.

E W, Diabetes mellitus (27), (Figure 6A) Severe ketonuria (65 to 11 grams of beta hydroxybutyric acid per day). There is a negative correlation (-0.45 ± 0.33) giving a negative $r = -3.6 \pm 2.4$, a value which has no meaning with respect to the hypothesis under discussion. The theoretical lines for $r=1$ or 2 bear no significant relation to the observed points. When ketone formation is calculated on the old 1:1 basis, there is found in this case on two occasions a ketone body excretion which exceeded the calculated ketone body formation. A similar observation was found in the case of Kramer.

Hypothesis of fat metabolism in the diabetic

In summary, it is apparent from this analysis that there is no significant relation between the ketone bodies utilized and the "antiketones" oxi-

dized. The hypothesis of obligatory coupling of ketone body-carbohydrate oxidation in the diabetic receives no support from the quantitative data in this series of cases of diabetes mellitus. It remains, therefore, to formulate an hypothesis which will be in conformity with the observations.

The following appears to fulfill these requirements:

Up to a certain level fat metabolism is complete and there is no ketonuria. Beyond this level fat metabolism is incomplete and part of the fat catabolized is excreted in the form of ketone bodies.

The relation of carbohydrate to fat metabolism is an inverse one: the greater the carbohydrate metabolism, the less is the fat metabolism. There is no fixed relation in the sense of a definite molecular ratio of ketogenic antiketogenic substances.

The hypothesis is elaborated in the form of a diagram (Figure 7) and an equation:

Let F = total fat catabolized in grams, mM, or equivalent mM of ketone bodies

U = total fat utilized in grams, mM, or equivalent mM of ketone bodies

U_0 = maximal aketonuric fat utilization

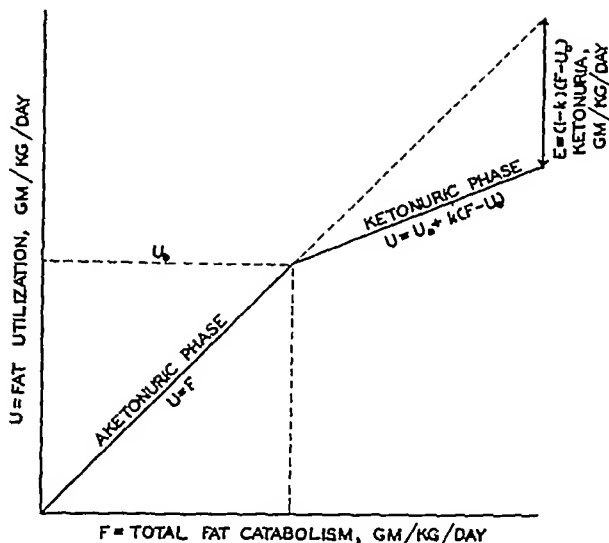


FIG. 7 SCHEMATIC REPRESENTATION OF FAT METABOLISM IN DIABETES MELLITUS

The figure embodies the hypothesis discussed in the text. The equations relating fat utilization and ketonuria to total fat catabolism are shown in the graph. F = total fat catabolized U = total fat utilized U_0 = maximal aketonuric fat utilization E = total urinary ketone bodies k = coefficient of excess fat utilization.

E = total urinary ketone bodies in mM of ketone bodies or the equivalent grams of mM of 'original' fat
 k = coefficient of excess fat utilization.'

In any given calculation all of the above terms must be expressed in the same units. The conversion factors necessary are

1 gram of fat

$$= \frac{1000}{870} = 1.15 \text{ mM of fat.}$$

$$= \frac{1000}{870} \times 12 = 13.8 \text{ mM of ketone bodies}$$

$$1 \text{ mM of ketone bodies} = \frac{1}{12} \frac{870}{1000}$$

$$= 0.0726 \text{ grams original " fat.}$$

$$= 0.0836 \text{ mM of original " fat.}$$

870 = molecular weight average fat (triglyceride) ⁵

The balance of the total metabolism not represented by carbohydrate or protein is, of course, represented by fat metabolism. Part or all of the total fats catabolized undergo a preliminary partial oxidation in the liver to acetoacetic or beta hydroxybutyric acids. The ketone bodies (or fat itself) are utilized in the peripheral tissue by both normal and diabetic subjects without chemical coupling with carbohydrate oxidation. Up to a certain point, catabolism keeps exact pace with the body's need for energy from fat and there is no ketonuria. We may express this by saying that $U = F$ and represent it on the diagram (Fig-

⁵ In accordance with the multiple alternate oxidation hypothesis these conversion factors are calculated on the assumption that 1 molecule of fatty acid yields 4 molecules of ketone bodies.

ure 7) as the first or *aketonuric phase* of fat metabolism. When, however, at a given state of activity the need for fat metabolism exceeds a certain *maximal aketonuric utilization value* (U_o) fat is catabolized in excess of utilization. The excess of fat catabolism over the aketonuric level is divided into two parts (1) the k th part is utilized as extra fat utilization, (2) the $(1-k)$ th part is excreted in the form of urinary ketone bodies. Therefore, in this second or *ketonuric phase* the fat metabolism is represented by the equation

$$U = F - E = U_o + k(F - U_o) \quad (2)$$

and the urinary ketone bodies are given by the equation

$$E = (1 - k)(F - U_o) \quad (3)$$

Application of the equation to the cases

The cases cited in Group I all had moderate to severe ketonuria. In other words $F > U_o$. Equation 2 may then be applied to the data. In the figures (1 to 6) (1) already given, the B segments show the data plotted in this way. In the conversion of the fat of the metabolic mixture to equivalent amounts of ketone bodies the ratio of four molecule of ketone bodies per molecule of fatty acid was assumed in accordance with the multiple alternate oxidation hypothesis. The statistical calculations are shown in the figures. In a series of cases (Group II) in which ketonuria was relatively low ($= < 5$ grams per day) or where there are but a few observations, the value of U_o has been calculated directly (Equation 2). These values have been included in the summarizing table (Table VI).

Inspection of Figures 1B to 6B* and of Table VI shows the following

1 The cases of Group I show in every case a high correlation between U_o and F , indicating that

* The labelling of the ordinates in these figures as "Ketone utilization" and "Total fat metabolism in ketone equivalents" does not imply that 100 per cent of the fat catabolized goes through the ketone body stage. The proportion which does is not known (*Cf* section on "Direct and indirect fat metabolism"). The terms "Fat utilization" and "Total fat catabolism" could just as well have been used as they are in Figure 9. The present terms are used here to emphasize the relation between fat catabolism expressed as ketones and potential ketone utilization.

TABLE VI

Summary of maximal basal aketonuric fat utilization in cases of diabetes mellitus

Case	Reference	Maximal aketonuric fat utilization in equivalents of ketone bodies	Coefficient of excess fat utilization
Group I		mM per kgm per day	
Cyril K	Gephart, Aub, DuBois and Lusk (25)	35	+0.41
Beattie B	Wilder, Boothby and Beeler (24)	34	+0.75
Kramer 740	Shaffer (22)	35	+0.28
E W	Joalin (23)	28	+0.75
Jervia B	Mosenthal and Lewis (27)	(47)*	-0.38
	Richardson and Ladd (26)	23	+0.84
Group II			
Ray H	Richardson and Ladd (26)	37	
Chris Q	Richardson and Ladd (26)	26	
Harold J	Richardson and Ladd (26)	37	
George H	Richardson and Ladd (26)	37	
Frank B	Richardson and Ladd (26)	37	
K A	McClelland, Spencer and Falk (28)	40	
Mean (11 cases)		34 ± 1.6 (S.E. of mean)	
Equivalent in grams of fat		2.5 ± 0.12	

* Excluded from basal mean on account of fever (102° F)

utilization of fats by the diabetic is a function of the total fat catabolism.

2 The values for U_o , the maximal basal aketonuric fat utilization, are all concordant (except E W, *cf* *infra*). The mean value was found to be 2.5 ± 0.12 grams of fat per kgm per day equivalent to 34 ± 1.6 mM of ketone bodies per kgm per day. This value is in essential agreement with the mean basal ketone utilization (32 ± 3.1 mM of ketone bodies per kgm per day, Table IV) found in the experimental animal by different workers and by different methods.

These findings are considered to constitute proof that the hypothesis concerning fat metabolism in diabetes mellitus already stated is in conformity with the quantitative data available in the literature.

Significance of the coefficient of excess fat utilization

It will be observed (Table VI) that the value of k , which we have called the coefficient of excess fat utilization, varies considerably among the six cases with sufficient data for its calculation. The significance of this is that some subjects (with high value of k) are able to utilize excess fat without an undue increase of ketonuria. Those

with low k values are only able to increase their total calories from fat at the cost of a relatively heavy increase of ketonuria. Whether this division of diabetics into two classes on the basis of their k values has any bearing on the problem of high fat versus low fat in the dietary cannot be said.

Fat metabolism in febrile severe diabetic (Mosen-thal and Lewis' E W)

This case had three features which are in contrast to those of the other cases

- 1 Fever, 102° F (Infected foot ulcer)
- 2 High aketonuric fat utilization level 46.6 ± 3.5 mM per kgm per day ketone bodies
- 3 Severe ketonuria (approximately 8 to 20 mM per kgm per day of beta hydroxybutyric acid)
- 4 Negative value of k , the coefficient of excess fat utilization

Since there is only one case in the series showing these peculiarities, discussion is speculative but it may be that this case represents an extreme type of fat metabolism, possibly characteristic of the febrile severe diabetic. The well known clinical fact that infection superimposed upon severe diabetes leads to sharp increase of ketonuria could then be reasonably explained as follows: unlike the afebrile diabetic who can increase his fat utilization above the aketonuric level (k positive) the febrile diabetic has a decreased tolerance for fat (k negative) as well as carbohydrate. In consequence, without appreciable change in total fat catabolism there is a sharp fall of fat utilization below the aketonuric utilization level accompanied necessarily by an increase of ketone body excretion.

Fat utilization in exercise

For basal conditions U_0 per unit of body weight will be constant and within limits should be the same for different subjects. As discussed in the introduction, the peripheral tissues are capable of oxidizing ketone bodies at a rate equivalent to 5 to 7 times the basal metabolic needs. It has also been well established that in the completely diabetic depancreatized animals or in severe diabetes mellitus there is essentially no change of ketone body excretion during exercise.

From equation (3) the ketone excretion is

$$E = (1 - k)(F - U_0)$$

Therefore, for two states of activity, basal and working, if we assume that k does not change, we get

$$\Delta F = \Delta U_0$$

The meaning of this is clear: during exercise the increase of utilization keeps pace with increased fat catabolism. This conclusion seems to be a simple explanation of the non increase of ketonuria in the diabetic during exercise, whereas, as has been previously pointed out, this non-increase is entirely incompatible with the obligatory coupling hypothesis.

Ketonuria in diabetes mellitus

We have preferred to discuss the problem of fat metabolism in diabetes mellitus in terms of utilization rather than in terms of urinary ketone bodies in order to emphasize ketone body oxidation rather than excretion. However, it is equally possible to show that the data on ketonuria in diabetes conform to the hypothesis. By the transformation of the equation (3) we obtain

$$E = (1 - k)(F - U_0)$$

By way of illustration, the data in one case are plotted in Figure 8 and the points may be compared with the line calculated using the constants already found. It is obvious that the relations hitherto described predict the course of ketonuria in the diabetic with precision.

It might also be emphasized that the information obtained from observations on ketonuria *per se* is limited. For ketone body formation may vary within wide limits up to utilization $= U_0$ without any ketonuria. Above this value the exact relation between formation and excretion cannot be determined unless the value of the 'coefficient of excess utilization' which varies widely from subject to subject is also known.

Direct and indirect fat metabolism

The proportion of the total fat metabolism represented by (a) preliminary partial oxidation in the liver to ketone bodies followed by oxidation of the ketone bodies in the periphery and (b)

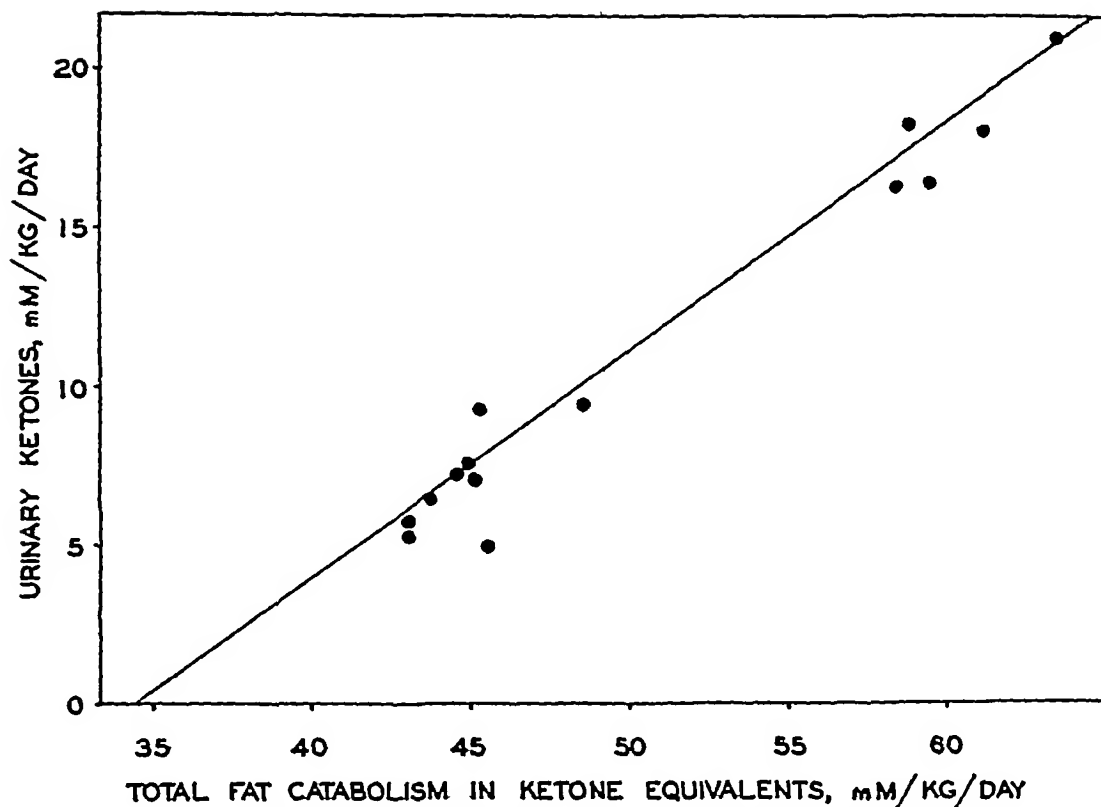


FIG 8 DIABETES MELLITUS, KRAMER (SHAFFER, 1922)

Urinary ketone body excretion as a function of the total fat catabolism. $E = (1 - k)(F - U_0)$
 Correlation = 0.99 ± 0.01 , $k = 0.28 \pm 0.03$, $U_0 = 35.3 \pm 1.3$ mM/kg/day

direct oxidation initiated and completed in the periphery, remains undetermined. There appears to be no doubt that ketone body utilization in the periphery may be sufficient to account for 4 to 6 times the basal requirements of the animal. But whether the liver does in fact partially oxidize such large amounts of fat is doubtful. For example, Stadie, Zapp, and Lukens (15a) found, with liver slices from depancreatized cats, ketone body formation equivalent to 1.3 mM per kgm per body weight per hour. But the total basal metabolism of the depancreatized cat, which must be largely that of fat, when expressed as ketone bodies is equivalent to approximately 4.5 mM per kgm per hour (calculated from data of Ring and Hampel (29)). Apparently only about 30 per cent of the total fat metabolism could be accounted for by the mechanism of preliminary oxidation to ketone bodies in the liver followed by complete oxidation in the periphery. In the normal fasted (2 to 4 days) cats they found still lower values, viz., about 10 per cent. In a series of fasted

phlorhizinized rats, hepatic ketone body formation was found to represent not more than 10 to 15 per cent of the estimated total basal metabolism (10).

The relation of hepatic and muscle respiratory metabolism

Another difficulty in the problem of fat metabolism which has not been sufficiently emphasized in the literature arises from a consideration of the oxygen requirements of the liver for the preliminary partial oxidation of fats to muscle substrates such as acetoacetate. From the observations of Barcroft and Kato (30) on the oxygen uptake of the gastrocnemius of the dog in the resting state and during stimulation at the rate of 60 times per second, the data shown in table VII have been calculated.⁷

⁷ Barcroft and Kato's results may be too high. (Compare, for example, Himwich and Rose (33).) However, cutting their values in half would not influence the main argument here.

TABLE VII

Observed muscle and calculated hepatic metabolism
of resting and exercising dog

For case 100 per cent preliminary hepatic partial
oxidation of fat to acetoacetic acid

	Observed oxygen uptake by muscle*	Calculated equivalent amount of acetoacetate oxidized by muscle	Calculated hepatic oxygen uptake required for production of acetoacetate from fat by partial oxidation in liver
	per gram of muscle micromoles per hour	per kgm body weight micromoles per hour	per gram of liver micromoles per hour
Resting	46	4.6	268
Exercising	166	16.6	968
Difference	120	12.0	700

Conversion factors

For total combustion in muscle 1 mole O_2 = $1/4$ mole
acetoacetate.

For partial oxidation of fat in liver 1 mole aceto-
acetate = 1.75 moles O_2 .

Assume

400 grams of muscle per kgm. body weight

30 grams liver per kgm. body weight

* Original data of Barcroft and Kato (30)

The table illustrates the expectation in the hypothetical case in which the entire muscular metabolism is dependent upon indirect fat utilization, i.e., one in which there is a preliminary partial oxidation of fat by the liver to acetoacetate. It will be noted that the increase, due to exercise, of the oxygen uptake per unit weight is some six times greater in the case of the liver than in the case of the muscle. Furthermore, these calculated hepatic respirations both in the resting and working state are greatly in excess of observed oxygen uptakes. For example, the observations of Staub (31) on perfused livers of dogs give a mean value of about 70 micromoles per gram of liver per hour for the oxygen uptake. In the intact cat Barcroft and Shore (32) found somewhat lower values. Innumerable observations of liver slices *in vitro* give a value of 75 to 100 μ M. per gram per hour. These values are about $1/3$ of the basal requirements and about $1/10$ of the requirements under exercise calculated in the table. It is of course, impossible to say that these intense hepatic respirations cannot be attained by the liver *in vivo*, but the marked discrepancy between the observed values (unless these are con-

sidered to be in gross error) and those calculated on the basis of complete indirect fat metabolism must be taken into consideration in interpreting the problem of fat metabolism.

The difficulties discussed in the last two sections can be overcome by supposing that a considerable part of the fat metabolism of muscle occurs directly, i.e., without preliminary hepatic partial oxidation to ketone bodies. The evidence on this point is not unequivocal. For example, Gemmell (34), who reviews the recent literature on this subject was unable to find any diminution of fat in the working muscle of the normal or phlorhizinized rat, and he concluded that when fat is used to supply energy for work in mammalian muscle, it is used indirectly. However, in view of the many observations of respiratory quotients ranging from 0.7 to 0.8 in the case of muscle with intact blood supply or isolated perfused muscle (33), or muscle equilibrated *in vitro*, the possibility of direct utilization of fat cannot be excluded. The extreme view, held by some physiologists that carbohydrate only can be oxidized by muscle appears no longer tenable.

Schematic representation of total metabolism in the diabetic

The relation of ketonuria to the fat metabolism and the total metabolism elicited by the study of these cases of diabetes mellitus can be incorporated into a scheme (Figure 9) which is intended to show the interrelations of these factors only in a general way. It is not a nomogram for application to specific cases. For simplicity assume a case to be without glycosuria deriving 20 per cent of the caloric needs from protein. Then

0.8 Total calories

$$= \text{Fat calories} + \text{Carbohydrate calories.}$$

The figure is based upon this equation and the necessary conversion factors. Several points of interest are shown by the diagram

1 Ketonuria is avoided if the fat catabolized is equal or less than about 2.5 grams per kgm. per day. It must be remembered that this value is for the resting state and may be higher during states of activity.

2 When the caloric metabolism is low keton

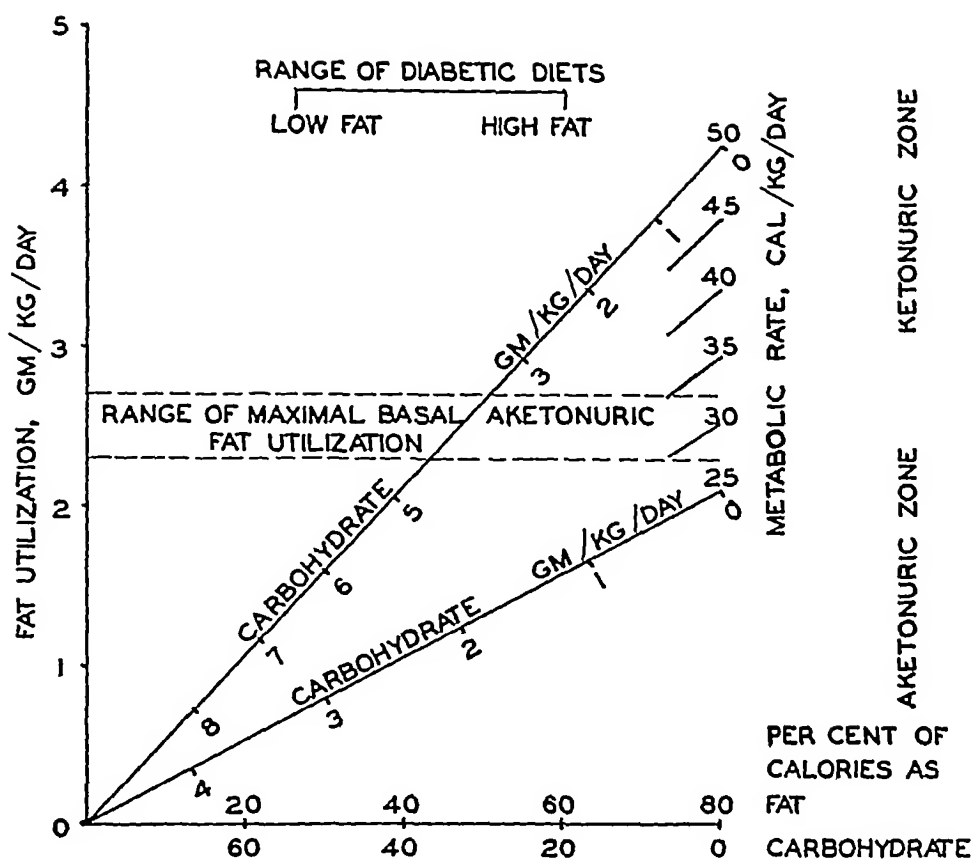


FIG 9 SCHEMATIC REPRESENTATION OF CARBOHYDRATE AND FAT METABOLISM IN DIABETES MELLITUS AND THEIR RELATION TO KETONE BODY EXCRETION

uria may be absent even when the carbohydrate metabolized is quite low

3 The greater the caloric needs, the greater must be the proportion of carbohydrate in the metabolic mixture in order to avoid ketonuria

4 The diets which through clinical experience have been recommended in the literature are, with respect to their proportions of carbohydrate and fat, essentially of the type which avoid ketonuria

4 The hypothesis of obligatory coupling of ketone body-carbohydrate oxidation in diabetes mellitus is not supported by the quantitative data in a series of cases

5 A simple hypothesis of fat metabolism in diabetes mellitus is presented and shown to conform to the observations in these cases

6 Other significant problems in fat metabolism are discussed

SUMMARY

1 The problem of fat metabolism in diabetes mellitus and its relation to ketonuria is reviewed and discussed

2 Evidence favoring the replacement of the successive beta oxidation hypothesis of fatty acids by the multiple alternate oxidation hypothesis is presented

3 It is shown that the diabetic animal utilizes ketone bodies in the periphery abundantly and independently of carbohydrate oxidation

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BASAL GASTRIC SECRETION AS A CLINICAL TEST OF GASTRIC FUNCTION WITH SPECIAL REFERENCE TO PEPTIC ULCER

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The use of gastric analysis as a diagnostic method has in recent years fallen under a cloud and, while descriptions of new modifications of test meals continue to appear, they receive at best a half hearted reception. The reason for this is not far to seek. Variations in gastric secretion are so wide in healthy people that very little specific diagnostic information can be elicited in disease. It is true, to be sure, that many patients with duodenal ulcer have a highly acid gastric juice but the limit attained by the normal stomach after the conventional stimuli is not transcended. (1) All one can say is that high acid is compatible with duodenal ulcer but not diagnostic. Very low acid or anacidity is strong evidence against duodenal ulcer, and this is perhaps the most valuable piece of clinical information to be obtained from gastric analysis. The anacidity which used to be thought so suggestive of cancer is now known to occur in many healthy elderly people (2), and the presence of acid even in large amounts by no means rules out neoplasms especially those forms originating in a peptic ulcer (3).

It occurred to us that potential differences in gastric secretion might be wiped out by powerful stimuli such as histamine which are in vogue in the clinic, and going a step farther we wondered whether measurements of the spontaneous gastric secretion obtained without the use of any stimulus might in disease show significant deviations from the normal and hence be useful in clinical diagnosis.

LITERATURE

Pavlov (4) working with dogs, found that in the interdigestive period no true gastric juice was secreted and that the reaction of the mucosa became alkaline. Beaumont years before had made similar observations in the case of his famous subject St. Martin. Carlson (5) on the other hand, pointed out that a continuous gastric

secretion occurred in some normal people and concluded that "complete rest of the gastric glands is an exceptional state in the healthy individual. These two views have led to a good deal of controversy and the subject is well analyzed in a recent paper by Pavlov's pupil Babkin (6). Much of the discussion has been of a quibbling sort, the advocates of the Pavlov position holding that any secretion of the apparently resting stomach is in fact due to unrecognized psychic influences or humoral stimuli from food in the intestines, and so forth. It is just because of the very fact that fasting secretion in different people represents the play of humoral and autonomic influences in the individual that its measurement may be of value in human pathology. Lim (7) and his associates made a systematic investigation of basal secretion in the dog. They found among other things that secretion continued after all extrinsic nervous connections to the stomach had been severed. However a great reduction to a low constant level followed section of the vagi, this suggested that reflex stimulation of gastric juice is mediated through these nerves. Necho-roshew (8) again with dogs, confirmed Pavlov's findings of intermittent secretion, but Krimberg (9) found a continuous secretion which he thought was kept up by a stimulating substance secreted by the juice itself. Roberts (10) made an extensive study of basal secretion in man. He pointed out that in a good many people there occurred at the end of an hour or so a sudden decrease of the "spontaneous" gastric juice to a very low level. He regarded basal secretion as due to a combination of "psychic" and "chemical" stimuli. Vandorfy (11) too found continuous secretion in man to be the rule and believes it to be a "physiological" process modified by psychic influences. There is a considerable literature on continuous gastric secretion during nocturnal sleep (12). All observers agree that during the hours of heavy sleep secretion is practically in abeyance in normal people but Henning and Norpoth (13) found that free secretion continued through the night in duodenal ulcer cases. Secretion was also present in patients with "gastritis" and "neurosis." Winkelstein (14) confirmed these findings. Many years ago Galambos (15) proposed the measurement of basal secretion as a clinical test of gastric function but his data were limited and seem to have attracted no attention.

Whatever the proper physiological interpretation of the views summarized above may turn out to be, it seems established that many people have a continuous secretion of gastric juice. This juice—"secreted by the stomach in the absence of all intentional and avoidable stimulation" (Lim)—represents the effect of nervous and

¹Supported by a grant from the Rockefeller Foundation.

humoral influences which are characteristic for the individual as we showed in a previous paper (16) where it was pointed out that repeated measurements of basal secretion in the same person under uniform conditions gave practically identical results. For these very reasons measurements of basal gastric secretion would seem useful in the clinical study of patients.

METHODS

The technique of measuring basal gastric secretion has been described in our previous papers (16). Briefly, the patient is at rest in bed overnight in the hospital and in the morning he is prepared as for a metabolism test. Without the use of any meal or stimulus a small tube is passed into the stomach and the fasting contents are withdrawn. Continuous aspiration is then kept up and the secretions are collected over successive ten-minute periods. Within forty minutes to an hour the stomach usually is found to be secreting at an approximately constant level and the acidity and the ten-minute secretory volume at this point are taken to represent the "basal secretion."

The question, of course, comes up as to whether the passage of the tube modifies true spontaneous gastric secretion, and this question, too, has received consideration in the literature, although no definite conclusion has been reached. There is evidence that gastric distention may stimulate a flow of juice (17), in other experiments it has been suggested that stimulation of the pharynx by the stomach tube reflexly provokes gastric secretion (18).

Our own observations indicate that passage of the tube may have a temporary stimulating or inhibiting effect in different people (or indeed no noticeable effect at all), as illustrated by the protocols in Tables I to V. Certainly no rule can be laid down. We also pointed out, as mentioned above, that repeated examinations of the same individual yielded a highly constant basal

TABLE I
Basal secretion (Case 93—normal)

Ten minute period number	Ten minute volume of secretion	Free hydrochloric acid	Total acid
Fasting	cc.		
1	5	52	67
2	3	58	72
3	7	94	104
4	6	102	113
5	3	96	115
6	6	70	85
*6	8	73	88

* The values for volume of secretion and total acid in this period are taken as "basal secretion."

Comment After the small amount of fasting contents was removed, there seemed to be a transient inhibition of acid in period 1 followed by stimulation in periods 2 to 4. Finally a basal level was reached in periods 5 and 6. Note low volumes of secretion throughout in contrast to high basal acidity.

TABLE II
Basal secretion (Case 88—normal)

Ten minute period number	Ten-minute volume of secretion	Free hydrochloric acid	Total acid
Fasting	cc		
1	9	3	5
2	7	3	35
3	9	31	63
4	7	22	48
5	7	10	35
6	9	0	17
*6	6	0	15

* Basal secretion

Comment After removal of fasting contents there seemed to be a transient stimulation of acid (periods 2 to 3) which then subsided to a basal level (period 6) with no free acid.

TABLE III
Basal secretion (Case 105—normal)

Ten minute period number	Ten minute volume of secretion	Free hydrochloric acid	Total acid
Fasting	cc		
1	30	0	8
2	8	0	6
3	4	0	6
4	7	0	10
5	10	0	10
6	4	0	13
*6	5	10	35
	Histamine		
7	12	12	40
8	23	63	72
9	20	72	86

* Basal secretion

Comment During the first few periods secretion seems to be inhibited. A small amount of acid subsequently is secreted (periods 5 to 6) but the volume of secretion is unchanged. After histamine there is a free flow of quite acid juice. Contrast this case with the preceding (Table II).

TABLE IV
Basal secretion (Case 95—normal)

Ten-minute period number	Ten-minute volume of secretion	Free hydrochloric acid	Total acid
Fasting	cc.		
1	45	0	4
2	10	8	27
3	22	5	21
4	18	17	37
5	15	35	56
6	7	35	60
7	8	29	56
*7	7	35	65

* Basal secretion.

Comment The fasting contents are often large in volume and low in acid due to admixture of saliva, duodenal contents, etc. In successive ten-minute periods there was first a free flow of juice of low acidity. During succeeding periods the volume of juice fell but the acid increased reaching a basal level 7 to 8 cc. per ten minutes with an acidity of approximately 60

TABLE V
Basal secretion (Case 106—normal)

Ten-minute period number	Ten-minute volume of secretion	Free hydrochloric acid	Total acid
Fasting	cc.		
1	60	44	60
2	8	44	60
3	5	34	58
4	6	29	58
5	6	38	67
6	5	38	67
*6	5	38	67

* Basal secretion

Comment In this case there seemed to be neither stimulation nor inhibition by passage of the tube. There is a steady practically uniform secretion from the start.

secretion and apparently pictured a certain type of stomach and autonomic organization.

Comparison of basal secretion with that obtained after histamine is of interest. Figure 1 shows a series of values for basal acidity in normal people and also the acidity attained in the same individuals after histamine stimulation. In almost all cases the acid was swept up to a high value and differences were much less clearly brought out than by the basal acidity

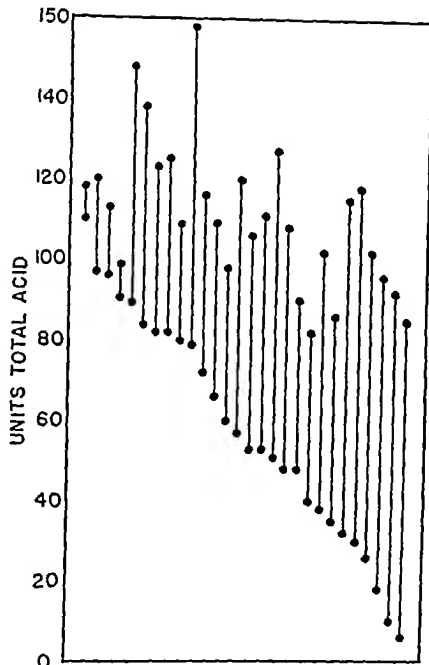


FIG. 1 COMPARISON OF BASAL GASTRIC ACIDITY AND ACIDITY AFTER HISTAMINE

Lower dots = basal acidity upper dots = level attained after standard dose of histamine in same individuals

Basal secretion in controls

In Figure 2 each solid black dot represents the total basal acidity in a different case, as measured by titration with phenolphthalein in the usual way. The patients were a miscellaneous hospital group, none with serious disease and none as far as we could tell with any disease of the gastro-intestinal tract. They serve as controls with which to compare the findings in peptic ulcer and other gastric disorders. Fourteen of these subjects had no free acid in the basal secretion but no patient is included who did not put out acid in response to histamine. No attempt was made in this small series to treat males and females separately but the values are plotted in relation to age. Points of note are the wide variations in basal acidity in

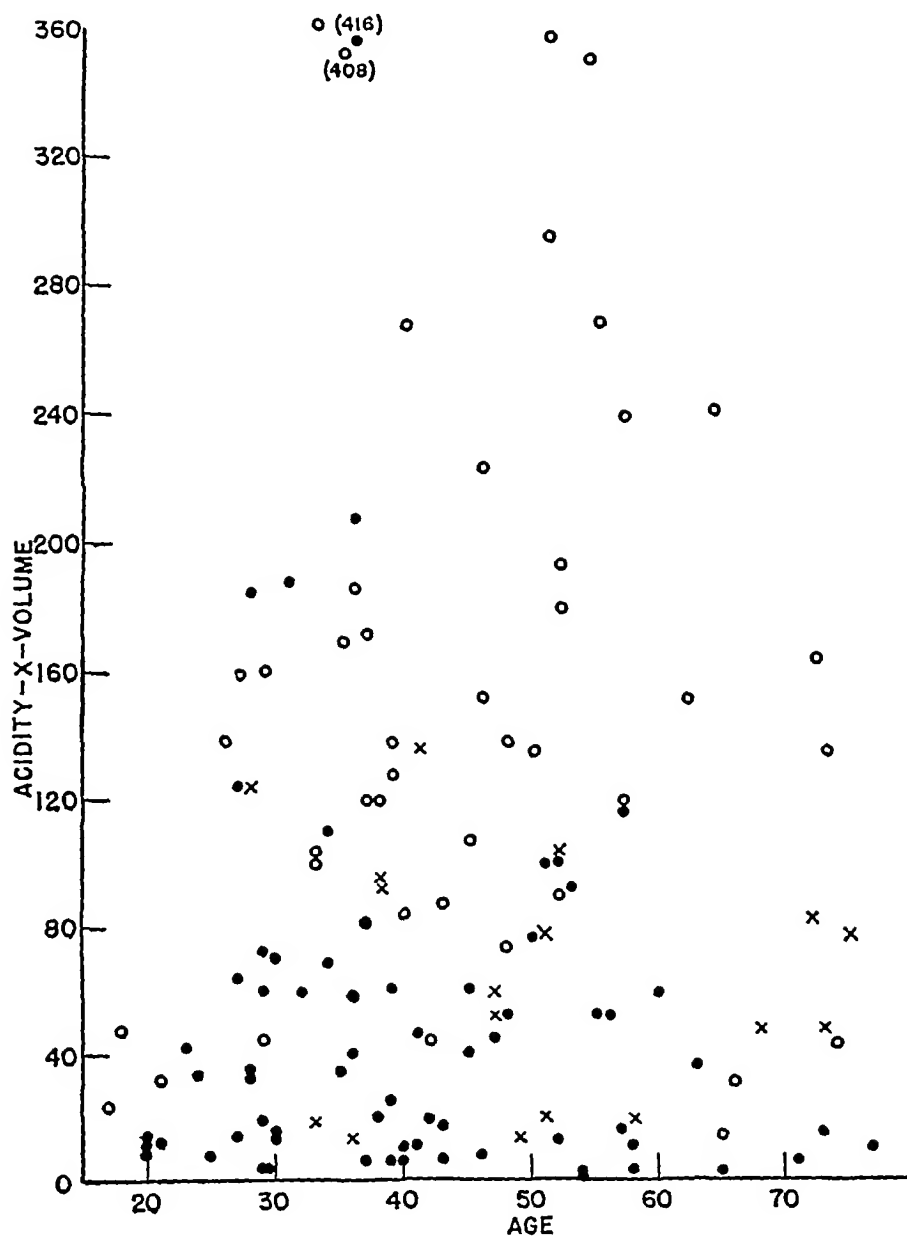


FIG. 4 RATE OF BASAL SECRETION
Same designations as in Figure 2

The wide variations in different people, which are to a large extent wiped out by histamine stimulation, are described

2 Similar measurements in a series of patients with peptic ulcer show that basal acidity with duodenal ulcer often exceeds the highest control values, hence this measurement has definite diagnostic value

3 Values for basal acidity in gastric ulcer cases

are similar to the controls and suggest that gastric and duodenal ulcer are fundamentally different disorders

4 Measurement of basal secretion is suggested as the simplest and most useful procedure in the study of clinical gastric physiology. If basal anacidity exists the test should be supplemented by histamine stimulation

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